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MEMOIRS OF THE DEPARTMENT OF AGRICULTURE IN INDIA

STUDIES IN BACTERIOLOGICAL ANALYSIS OF INDIAN SOILS

No. 1

1910–1911

BY

C. M. HUTCHINSON, B.A

Imperial Agricultural Bacteriologist, Pusa

AGRICULTURAL RESEARCH INSTITUTE, PUSA

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STUDIES IN BACTERIOLOGICAL ANALYSIS OF INDIAN SOILS. NO. I, 1910-1911.

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THE science of soil bacteriology has now reached a stage at which certain fundamental conceptions are generally recognised as being axiomatic; the principal one upon which the methods of dealing with the subject depend is that which involves the recognition of soil organisms as necessary intermediaries between the plant and the nitrogen of the soil. These organisms may be divided into two groups with reference to this central idea, namely, those which convert the nitrogen of the organic matter in the soil into ammonia and nitrates, and those which take free nitrogen from the air and bring it into a state of combination as organic matter, thus adding to the nitrogen supply in the soil; associated with the former are many bacteria and moulds, which, by their action upon such non-nitrogenous bodies as cellulose, help to break down the complex tissues of root residues and organic manures in the soil. At the same time it is necessary to take into account the action of those organisms which under certain conditions cause loss of nitrogen by reduction of its compounds, giving rise to the phenomena of denitrification. It may then be said that investigations in soil bacteriology are directed mainly towards determining in what manner soil nitrogen is dealt with by the micro-organisms in any soil under examination, and particularly under what conditions of moisture, temperature, and aeration these changes take place; such investigations may be conveniently referred to as methods of biological analysis of

soils, and I propose to give some account of the results so far obtained at Pusa by the application of such methods of analysis to Indian soils.

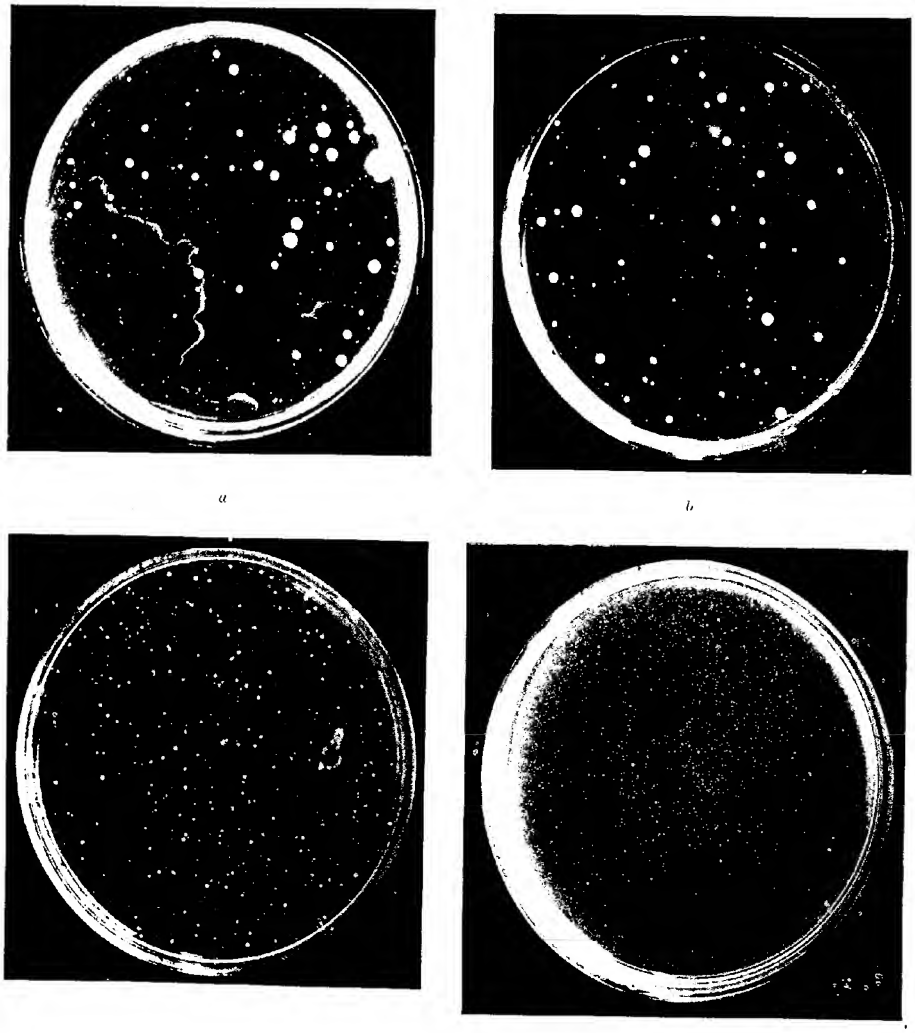
The biological analysis of a soil involves—

1. Determination of number and kind of organisms present.
2. Estimation of their physiological activity under varying conditions.

The first determination is made by plating, and the second by obtaining pure cultures of the various organisms, or more usually by ascertaining by approximate methods the collective effect of the various organisms present. There can be no doubt, however, that in order to arrive at a complete understanding of the various processes of decomposition effected by soil organisms, it will be necessary to ascertain the characters and physiological functions of individual species alone and in combination with others, and the work of Winogradsky, Omélianski, Warington and others, in determining the characters of the organisms of nitrification, well illustrates the value of such methods.

At the present time, however, there are many reasons for not attaching too much importance to the method of plating and enumeration of bacteria as a means of obtaining reliable information as to the bacterial content of any soil. In the first place, the method is unreliable on account of the difficulty of obtaining representative samples of soil, and although this may be overcome to some extent by laborious reduplication, further difficulties arise in connection with the condition of the soil with regard to aeration and moisture at the time of taking the samples, and the variation in the soil flora taking place in the period of time elapsing between this moment and the making of the plate. It was found, for instance, that two samples of the same soil taken within ten days of one another from an experimental plot on the farm of the Agricultural College at Cawnpore, gave numbers in the first sample of 500 millions per gram and in the second of $2\frac{1}{2}$ millions. These samples were taken on the 2nd February 1911, and the 10th February 1911, respectively, the

PLATE I.



(*a*) Air-dry Soil + 50% water.
(*b*) ,, ,, + 25% ,,
(*c*) Air-dry Soil + 10% water.
(*d*) Air-dry.

differences being apparently due to the fact that 0·1 inch rain had fallen, after a drought of eleven weeks, 48 hours before taking the first sample; no more rain having fallen between the date of the first sampling and that of the second, the bacterial numbers fell to the lower figure. A further and equally serious variation may be introduced by alteration of the moisture content of the soil after taking the sample; a sample of Pusa soil was air-dried for 24 hours at an average temperature of 30° C. and divided into four portions, to three of which different amounts of water were added, 10%, 25%, 50%; the fourth remaining air-dry and containing 1·5% moisture; these portions were incubated at a temperature of 30° C. and plated after 48 hours. The photograph of the four plates (Plate I) shews the difference in numbers of organisms present as a result of this variation in treatment.

Rémy has demonstrated (Cent. f. Bakt. 2nd Abt. 8. 1902) that plate counts of bacteria show no direct relation to the nitrifying or ammonifying power of the soil from which the plates were made, and Löhnis (Cent. f. Bakt. 2 Abt. 12. 1904), is of the same opinion. The reasons for such discrepancy are many, one of the most important being the fact that upon introducing a heterogeneous mixture of organisms into a culture medium such as agar or gelatine, certain rapid growing species obtain a hold to the detriment or exclusion of the remainder, and, even although this condition may be minimised by the use of high dilutions and of such media as the synthetic agar recommended by Lipman and Brown (Cent. f. Bakt. 25 Bd. 1909), it has been my experience at Pusa that no method of plating has given adequate representation of the various organisms present in a soil sample, many of which appear only after prolonged incubation, during which the spreading of certain colonies may have occurred to such an extent as to lead to the complete suppression of numerous individuals.

In biological analysis, therefore, quantitative results obtained by plating have a value which is limited by the above considerations, except when pure cultures are involved, or when plates

made from the same soil under different conditions of treatment show variations in the number of colonies of the same species; and even this variation, it must be recognized, may be due to interference produced by partial or complete suppression of some other species normally present. As an illustration of the differences produced by variations in the method of plating, the following examples may be cited.

Pusa soil. Custard apple plot; Botanical Section; sample from 2nd to 6th inch—medium, synthetic agar as recommended by Lipman and Brown, of following composition :—

Synthetic Agar.

Dextrose	10·0 Gms.
Di-potassium phosphate	0·5 ,,
$MgSO_4$	0·2 ,,
KNO_3	0·5 ,,
Agar	20·00 ,,
Tap water	1000 c. c.
	+5 Fuller's Scale.

Incubated at 20°C.

Soil emulsified and diluted in 0·75% NaCl.

TABLE I.

EFFECT OF VARYING DILUTIONS ON PLATING.

	Method of Dilution.	Dilution.	Colonies in duplicate plates after 3 days.	Average No. of colonies per plate.	No. of colonies per gram of soil, calculated.
1	40 gms.—400 c.c. H_2O ... 10 c.c.—100 c.c. H_2O ... 1 c.c. for inoculation ...	1 in 100 ...	a. 11,200 ... b. 9,600 ...	10,400	1,040,000
2	40 gms.—400 c.c. H_2O ... 10 c.c.—100 c.c. H_2O ... $\frac{1}{2}$ c.c. for inoculation ...	1 in 200 ...	a. 6,096 ... b. 5,120 ...	5,608	1,121,600
3	40 gms.—400 c.c. H_2O ... 10 c.c.—100 c.c. H_2O ... $\frac{1}{10}$ c.c. for inoculation ...	1 in 1,000 ...	a. 1,255 ... b. 1,335 ...	1,295	1,295,000
4	10 gms.—250 c.c. H_2O ... 1 c.c.—250 c.c. H_2O ... 1 c.c. for inoculation ...	1 in 6,250 ...	a. 420 ... b. 330 ...	375	2,358,750
5	10 gms.—250 c.c. H_2O ... 1 c.c.—250 c.c. H_2O ... $\frac{1}{2}$ cc. for inoculation ...	1 in 12,500 ...	a. 185 ... b. 200 ...	207	2,587,500
6	10 gms.—250 c.c. H_2O ... 1 c.c —250 c.c. ... 1 loop for inoculation ...	1 in 125,000	a. 3 ... b. 3 ...	3	375,000

TABLE II.

DILUTIONS IN WATER AND SALT SOLUTION COMPARED.

	Method of Dilution.	Dilution.	Colonies in duplicate plates after 3 days.	Average No. of Colonies per plate.	No. of Colonies per gram of soil.
1	10 gms.—250 c.c. H_2O ... 2·5 c.c.—100 c.c. H_2O ... 1 c.c.—100 c.c. H_2O ... 1 c.c. for inoculation ...	1 in 100,000	a. 22 ... b. 31 ...	26	2,600,000
2	10 gms. in 250 c.c. normal salt solution ... 2·5 c.c.—100 c.c. normal salt solution ... 1 c.c.—100 c.c. normal salt solution ... 1 c.c. for inoculation ...	1 in 100,000	a. 38 ... b. 48 ...	43	4,300,000

From these examples the necessity for selecting the dilution appropriate to the soil is apparent; in Nos. 1, 2, 3, the number of colonies per plate is too great to allow of the development of all the forms present, whilst in No. 6 the dilution is so high as to result in imperfect sampling and lack of representation from an opposite cause.

The use of normal salt solution further alters the apparent number of bacteria per gram of soil, and emphasizes the necessity for adopting standard conditions in plating, and for not placing too much reliance on numerical results obtained by this operation. On agar the tendency to spread is very marked, so that after a few days, certain organisms will cover more than half a 9 c.m. petri dish to the complete suppression of others which might have appeared later. As it is impracticable in the plains of Bengal to use gelatine plates during the hot weather, *i.e.*, from the beginning of March until the end of October, except under very restricted conditions,* the use of the plate method for determining the numbers of organisms per unit of soil is attended with drawbacks which limit its use very considerably. Nevertheless it has been found possible to utilize it for making certain quantitative determinations, mainly in connection with the effects of

* I have not been able to prepare a completely sterile gelatine medium with a high melting point, by the method recommended by J. Forster (Cent. f. Bkt. XXII Bd. 1897—pp. 341-3).

various methods of treatment upon the composition of the soil flora, but it appears most probable that dilutions sufficient to reduce the colonies on a 9 c.m. plate to less than about 50 in number, do not as a rule result in the inclusion of all the species present in the soil.

Many other soil bacteriologists have demonstrated the impossibility of obtaining representative plates by the use of small samples of soil, such as one gram, and it is now very generally recognized that a nearer approximation is arrived at by making an emulsion with a comparatively large quantity such as 10 grams, or even 100 grams, and subsequently diluting this extract. Löhnis (Cent. f. Bakt. II Abt. 14—1905) has pointed out the unreliability of the plate method as a means of arriving at an estimate of the number and kind of organisms present, and of drawing inferences as to the relation between their number and the physiological activity and fertility of the soil; in common with other investigators he places more reliance on the method introduced by Rémy (Cent. f. Bakt. II Abt. 8—1902) of observing the physiological activity of the soil complex by introducing portions of the soil into solutions of known composition. In recent work this method has been largely adopted by many investigators, and the modification proposed by Lipman, who used soil infusions in place of the soil itself has been shewn to be of great value by Bühlert and Fickendey (Cent. f. Bakt. II Abt. 16—1906).

Greig-Smith has demonstrated (Linn. Soc. N. S. W. 1910) the probability of the existence of bacterio-toxins in soil, and points out that their interference with bacterial growth may be measured by plate counts, but in this case the plates were not made direct from soil dilutions. Such toxins are destroyed either partially or completely, or perhaps rendered insoluble, by various agencies such as sun-light, heat, or by exposure to air or to the action of certain antiseptics and chemical reagents. If we are prepared to assume the presence of such toxins in soil, and very many ascertained facts in connection with soil bacteriology support this view, we find the use of plating as a method of biological

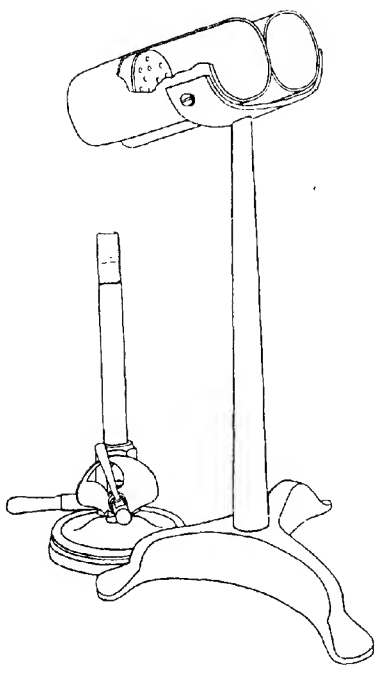


Fig. 1.

Plug Sterilizer.

analysis still further complicated by the intervention of these agencies, which may come into operation during the processes of sampling and preparing the soil for use as an inoculum. It may be pointed out that the use of a soil emulsion may carry into the culture medium a greater amount of any soluble bacterio-toxins present in the soil sample than would the small soil inoculum itself. It appears unfortunate that many investigators have published results of soil plating without providing information as to the method of preparing the samples, or of the method or extent of dilution. I have found at Pusa (cf. Table II, p. 5) by comparative trials that the use of normal salt solution (0·75 per cent. NaCl) in many cases considerably modifies the number of species appearing on a soil plate, as compared with those present when water alone has been employed; in the majority of cases this modification consists in the earlier appearance of colonies of the motile forms, and suggests the opinion that these latter are more susceptible to osmotic disturbances than the non-flagellated organisms.

A characteristic feature of plating and other culture work at Pusa is the tendency to contamination by air-borne organisms, which very much exceeds any such liability in more temperate climates. The principal source of such contamination is the presence of mould spores, mainly of Penicillium, which constantly appear on any plate, although not present in the culture originally. These spores will germinate also on the outside surface or in the interior of cotton-wool plugs, and, pushing their hyphæ through the latter into the interior of a tube culture, form fresh spores which drop on to and infect the medium. The ordinary processes of steaming during sterilization of tubes or flasks of medium are quite inadequate for freeing the interior of plugs from such spores, and it is necessary to sterilize them separately in the autoclave. In making transfers I have found it necessary to utilize a special device for retaining sterility of the plugs; this consists of a pair of short brass tubes mounted on an iron stand, Fig. I, and kept at such a temperature by means of a Bunsen burner as to produce slight browning of the cotton-

wool on introducing the plug into the mouth of the tube; in making transfers the plugs are removed with heated forceps, inserted into the plug sterilizer, the inoculation made, and the plugs replaced, their temperature in the meantime having been raised sufficiently not only to destroy any incident spores, but also any hyphæ which may have developed in the interior of the plug and which might not be destroyed by superficial singeing; at the same time the plug is hot enough to sterilize the mouth of the culture tube when reintroduced into the latter.

Organisms found in Pusa Soil.

This soil is a highly calcareous sand containing about 2·0% organic matter; its chemical and physical characters have been described in a Memoir of the Department of Agriculture in India, Chemical Series (Water requirements of Crops in India—J. W. Leather). Plate cultures were made on agar, ordinary and synthetic, the former prepared with Lemco and containing 2% agar, the latter containing

Synthetic Agar:

Gms.	
10·0	Dextrose.
0·5	Dipotassium Phosphate
0·2	Mag. Sulphate.
0·05	Pot. Nitrate.
20·0	Agar.
1000 c.c.	Water.

made up to + 5° Fuller, according to formula of Lipman and Brown.

Method of dilution—

10 gms. soil in 250 c.c Normal salt solution.
1 c.c. in 250 c.c.
1 c.c. as inoculum in 10 c.c. Agar
Dilution 1 in 6250.

The plates were incubated at 22°C. and counts made up to one week.

The following organisms were found on ordinary agar; + 5° Fuller :—

Bac. Mycoides.
„ Subtilis.
„ Mesentericus.

Bac Fluorescens Liquefaciens.
Sarcina lutea.
„ Alba.
„ Brown.
„ Pink.
Micrococcus Candicans.
Motile Bacteria—Rods 1·6μ × ·6μ, forming yellow colonies, round, raised.
„ „ Rods ·8μ—1·6μ × ·4μ—·5μ, forming Blue-Fluorescent colonies, round, flat.
Moulds—Penicillium, etc.

On synthetic agar the following additional organisms appeared :—

Streptothrix—White.
Yeasts (*a*) Pink, and (*b*) White.
Fungi—Trichosporium, Cladosporium-Aspergillus.
Short rods forming white, slimy, tenacious colonies, and similar in morphological characters to root nodule organisms of Dolichos Lablab; subsequently gave similar cultural characters on Ash-Maltose Agar.

Bac. Prodigiosus was not found in Pusa soil, although isolated from samples from Goa and Assam; varieties of Streptothrix were noticeably absent.

Tests were made of the relative ammonifying power of the above organisms, with a view to determining their action on soil humus as antecedents to nitrification.

Pure cultures on agar were seeded into Rémy solution (one loop (2 m.m.) in 50 c.c.), and distilled with magnesia, after incubating for a week at 22° C.

Two alternative methods were adopted in duplicate; in No. I the solution was placed in 25 c.c. Erlenmeyer flasks; in No. II ignited sand was added and arranged as a slope emerging above the solution.

In each case the inoculum was one loop of culture, 48 hours' growth on Agar at 30° C. 50 c.c. of the Rémy solution was distilled with magnesia, after seven days' incubation at 30° C.

Comparative Table of the Ammonifying Power of Bacteria in Rémy's solution and Rémy's solution with sand slope.

TABLE III.

	Milligrams of nitrogen as ammonia after seven days.	
	In Rémy's solution.	With sand slope.
B. Subtilis	6·1	6·9
B. Mycoides	9·1	11·1
B. Mesentericus	8·4	8·9
Sarcina Lutea	6·0	5·6
Sarcina (Brown)	3·5	5·3
Sarcina (Pink)	4·8	5·6
Sarcina Alba	5·4	6·0
Bluish fluorescent colony	3·1	2·1
Streptothrix (White chalky)	7·0	14·1
B. Megatherium	13·4	18.1
B. Prodigiosus	12·3	6·8
B. Fluorescens liquefaciens	7·4	7·2
B. Subtilis + B. Mycoides	7·70	7·98

B. prodigiosus was also tested and shewed a remarkable reversal of the almost universal increase of ammonia formation on the sand slope as compared with the non-aerated solution. It is, of course, unlikely that figures obtained in this way are of any absolute value as indicators of the relative ammonifying power of the various organisms compared, although duplicate determinations confirmed these results very closely; as is well known, the physiological as well as the morphological characters of bacteria vary profoundly when kept in pure culture, and would not be likely to bear any definite relationship to their properties in the soil. In connection with determinations of ammonifying power of various bacteria, whether in pure culture or in soils, an experiment was made to ascertain under what conditions the most reliable results could be obtained, as it appeared probable that the method of Rémy, of inoculating direct into a peptone solution, does not approximate to conditions obtaining in soil. The experiment was arranged as follows :—

Ammonification in Rémy Solution.

Three different proportions of soil to solution were used :—

I.—1 gram soil + 25 c.c. Rémy solution.
II.—10 „ „ +25 c.c. „ „
III.—200 „ „ +25 c.c. „ „
} Made up to 100 c.c. with water.

In I and II air was aspirated through the solutions, and in III over the moistened soil contained in a Woulff's bottle, and led through $\frac{N}{10}$ sulphuric acid; about 5 litres per diem being passed. After 9 days the solution and soil were distilled with magnesia.

The total amounts of nitrogen as ammonia given off and absorbed in the standard acid during the nine days were—

I.—0·6 Mgm. N.
II.—1·33 „ „
III.—1·33 „ „

The amounts of nitrogen as ammonia found on distillation, after 9 days' aspiration, were—

I.—30·8 Mgm. N.
II.—28·7 „ „
III.—23·85 „ „

The decided difference between the amount of ammonia formed in solution and in soil in this experiment suggests that the use of the former as a means of measuring the ammonifying power of any specific soil sample, or of any specific organism or combination of organisms is not to be relied upon except under special conditions, and with regard to the following considerations; it seems probable that on introducing a soil inoculum into Rémy solution the organisms contained in the soil will multiply up to the limit of the medium, this limit depending on the volume, concentration, and composition of the latter, and also upon its gas content, so that no indication will be obtained as to the ammonifying power of the soil, except during a short time immediately after inoculation, the duration of which it would be difficult, if not impossible, to determine; this is well shown in the above experiment where 1 gram of soil produced as much ammonia as 10 grams from the same amount of Rémy solution in the same time, the period being nine days. In the case of such a solution, therefore, the time factor may be regarded as of prime importance, and this was found to be the case by Russell and Hutchinson in their experiments on increased

ammonification in toluened soils (Journal of Agri. Science, Vol. III, Part 2); in this case the figures given for ammonia production on p. 131 illustrate this point, the significant difference between the toluened and untreated soils only occurring between the 12th and 24th hours, before and after which it does not appear, nor indeed do the figures obtained by this method help to substantiate the protozoal theory, as it will be seen (p. 131 loc. cit.) that in Rémy solution the rate of ammonification is not proportional to the number of organisms found by plating, although in soil as a medium the differences produced by toluening, both in number of organisms and in rate of ammonification are very great.

A further experiment was made as follows :—

Flask A	Rémy Solution	25 c.c.	+	H_2O	25 c.c.	+ 1 gm.	soil.
B	„	„	25 c c.	+	„ 75	+ 1 gm.	„
C	„	„	25 c.c.	+	„ 25 c.c.	+ 10 gms.	„
D	„	„	25 c.c.	+	„ 75 c.c.	+ 10 gms.	„
E+F	„	„	25 c c.	+	167 gms soil.		
G	„	„	25 c.c.	control uninoculated.			

The soil in E & F contained 16 per cent. moisture after addition of the Rémy solution, and this was maintained by periodic weighment and addition of water, this percentage of moisture having been found to be the optimum for ammonification in this soil. After seven days incubation at 30°C. A, B, C, D and G were distilled with magnesia into $\frac{N}{10}$ acid.

In E half the soil was distilled direct with magnesia (result under E_1) and the other half acidified with dilute HCl-water added to make a volume of 500 c.c. shaken for half an hour, allowed to settle, and 250 c.c. decanted and filtered and distilled with magnesia (result under E).

In F, 475 c.c. of water were added, making a total volume of 500 c.c. of which 300 c.c. were taken for distillation.

The total nitrogen in 25 c.c. of the Rémy solution was determined.

TABLE IV.

Total Nitrogen in 25 c.c. Rémy Solution = 37·35 mgms.

	Treatment.	After seven days.	
		Mgms. of N as NH_3.	% of total Nitrogen ammonified.
G	Control—no inoculum	0·7	.....
A	25c.c. Rémy + 25 c.c. H_2O + 1 gm. soil .. .	26·4	70·7
B	Do. + 75 c.c. H_2O + Do.	30·1	83·3
C	Do. + 25 c.c. H_2O + 10 gm. soil	29·1	77·9
D	Do. + 75 c.c. H_2O + Do.	30·95	82·8
E_1	Do. + 167 gm. soil distilled direct	4·5	12·05
E_2	Do. + 167 gm. soil distilled after acidifying with HCl shaking and filtering	10·2	27·3
F	25 c.c. Rémy + 167 gm. soil distilled after shaking and filtering	1·4	3·7

These results confirmed the previous conclusions as to the want of difference measurable in Rémy solution between various soil samples, and also emphasized the necessity for adopting a standard method of estimating the ammonia present in soil.

In further experiments depending upon determination of ammonia formation, either in peptone media or from organic matter present in or added to soil, I have mainly depended upon the use of pure cultures of B. subtilis, finding less variation in its ammonifying power under varying conditions of culture than in the case of B. mycoides, or B. mesentericus; it will be seen from Table III that differences of aeration have very little effect on its ammonifying power as compared with that of B. mycoides, B. megatherium, or B. prodigiosus.

Sterilization of Soils.

As a preliminary to most experimental work on biological analysis of soils, it is frequently necessary to prepare sterile soil. It may be of interest to reproduce here the various results obtained at Pusa in connexion with the complete or partial sterilization of soil. The sterility of the samples after treatment was

tested by plating, as well as by inoculation in peptone, as it was desired to discover what species survived the various methods of treatment.

Some of the results were obtained in connexion with a set of experiments designed to obtain information as to the bearing of the theory of protozoal intervention in partially sterilized soils upon special methods of cultivation in India; further reference to them will be made in dealing with this investigation.

Owing to the necessity in many experiments of working with soil media containing regulated quantities of water, it was found useful to ascertain the effects of sterilization by heat upon the amount of moisture remaining in the sample after treatment. These results are given in Table V.

TABLE V.

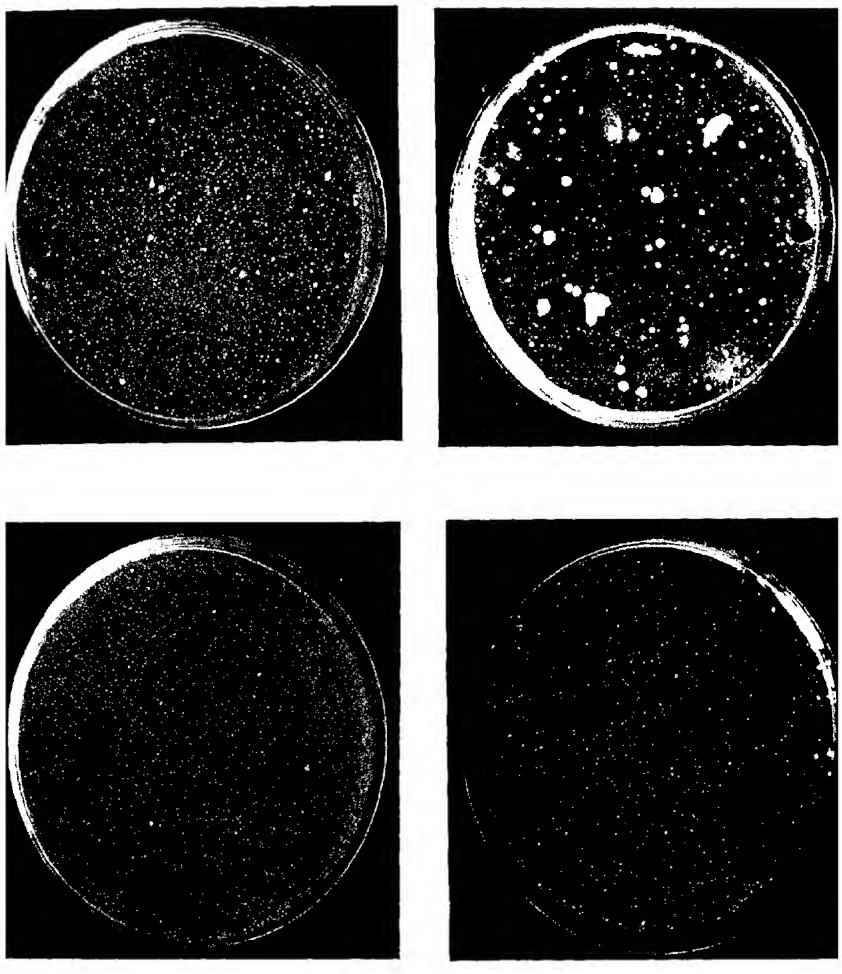
GAIN OR LOSS OF WATER FROM SOILS AND SOLUTIONS DURING STERILIZATION IN THE AUTOCLAVE.

	Original water content.	Gain or loss in c.c. after sterilization in the autoclave at 130° C. for ½ hour.		
		After 1st.	After 2nd.	After 3rd.
400 gms. soil airdry ...	2%	+ 1 c.c.	.....	.....
400 gms. soil ¼ saturated ...	10-12%	- 13-15 c.c.	- 18-19 c.c.	- 23-24 c.c.
400 gms. ½ saturated ...	20-24%	- 22-24 c.c.	28-29 c.c.	32-33 c.c.
400 gms. soil ¾ saturated ...	26-32%	- 28-29 c.c.	- 32-35 c.c.	- 40-41 c.c.
100 c.c. Oméliansky's solution	.....	- 10 c.c.	.....	.....
100 c.c. Peptone Broth ...	.....	- 10 c.c.	.....	.....
50 c.c. Oméliansky's solution	.....	- 5 c.c.	.....	.....

EFFECTS OF VARYING METHODS OF STERILIZATION UPON BACTERIAL CONTENT OF PUSA SOIL.

The soil was taken from the Botanical area—Custard apple plot—1st six inches. The sample was sifted through 3 mm. sieve, and 30 per cent. sand was added to it and thoroughly mixed. This addition of sand was made in order to avoid the tendency to

PLATE II.



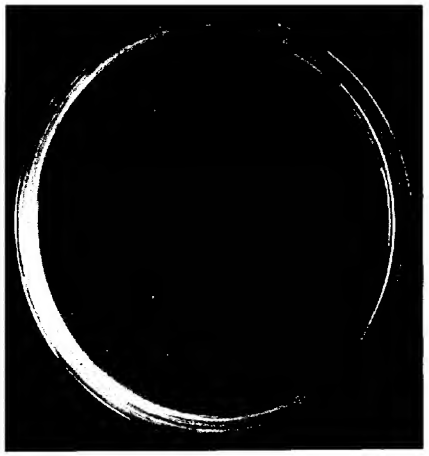
d

EFFECT OF VARYING STERILIZATION.

(*a*) Control unsterilized.
(*c*) Water oven 60°C.
(*d*) Koch 100°C. 3 hours continuous.
(*e*) 5% Toluene.

PLATE III.

f



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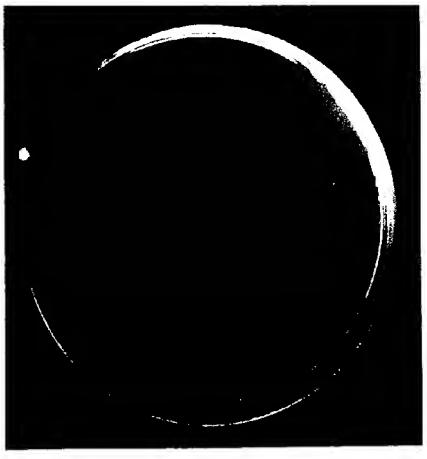

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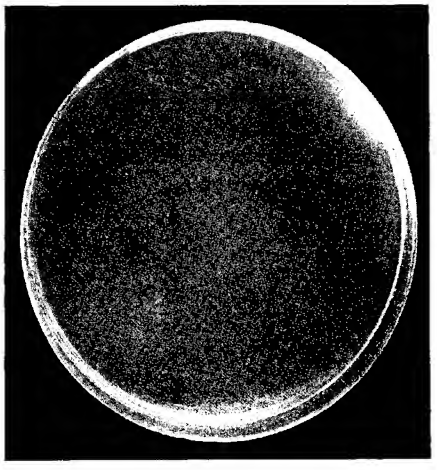


EFFECT OF VARYING STERILIZATION.

(*f*) Koch 100° 1 hour three times.
(*g*) Autoclave 130°C. ½ hour three times.
(*h*) Autoclave 130°C. ½ hour once.
(*i*) Hot air 150°C. ½ hour three times.

shrinkage and cracking displayed by this soil when used for pot cultures. 800 gm. portions of soil were placed in 48 ozs. bottles plugged with cotton-wool, water content made up to 20 per cent. and submitted to treatment. After treatment the soil was plated in Synthetic Agar (10 gms. soil in 250 c.c. Normal salt solution : 1 c.c. in 250 c.c.—1 c.c. inoculum) and incubated at 20° C. for five days.

TREATMENT.

a. Control unsterilized.

b. Unsterilized + 0·1 gm. oilcake per 100 gm. soil.

c. Heated to 60° C. in water oven for 6 hours on 3 consecutive days.

d. Heated in Koch at 100° C. for 3 hours.

e. 5 per cent. Toluene for 3 days—Toluene then evaporated for 6 hours.

f. Heated in Koch at 100° C. for 1 hour on 3 consecutive days.

g. Heated in autoclave at 130° C. for ½ hour on 3 consecutive days.

h. 0·1 per cent. oilcake mixed with soil and then heated as in *g*.

i. Heated to 150° C. in hot air oven for ½ hour on 3 consecutive days.

Photographs of these soil plates are given in Plates II and III.

From the results it will be seen that intermittent heating in the Koch steamer for one hour on three successive days is not sufficient to produce sterility in soil under the conditions of the experiment ; it appeared probable that this was due to the large amount of soil under treatment, and a further experiment was made in which similar treatment was given to smaller portions (20 gms.) of the same soil placed in 250 c.c. Erlenmeyer flasks. Details of this experiment are given with the results obtained in investigating the special problem of "weathering" (pp. 48-50), from which it will be seen that sterility was not obtained in the 20 gm. portions by two heatings in the Koch,

although exposure to formaldehyde vapour for 24 hours was completely effective. It is obvious therefore that a temperature of 98° C., although applied intermittently on successive days, is not sufficient to sterilize 20 gm. portions of soil containing the large number of resistant organisms characteristic of Pusa soil. It will be noticed, however, that the anaerobic organisms are completely destroyed by this temperature (results of plating under anaerobic conditions, p. 53), and further that the surviving organisms generally belong to classes shewing greater individual ammonifying power than those which are eliminated by this amount of heating, this being also the case with less drastic methods of sterilization, as was shewn by Russell and Hutchinson (Jour. Agri. Sci., Vol. III, Part 2, p. 133). These investigators, however, are of opinion that the apparent increase in ammonifying power of the surviving organisms, in the case of toluene treated soil, is not due to survival of more effective individual numbers of the soil flora, as they have found that individual species suffer loss of physiological activity after treatment with toluene; I have found a similar persistence of modified activity in succeeding generations in the case of B. prodigiosus which, on treatment with ultra-violet rays from a mercury vapour quartz lamp, of insufficient duration to kill the organisms, lost their pigment-producing power, which was only regained after several transfers.

It may be of interest to give here the result of a separate experiment made to determine the efficiency of thymol as a sterilizing agent in soil. In this experiment 20 gms. of soil to which was added 0·02 gm. of cake (mustard) was moistened with 2·5 c.c. of a saturated solution of thymol in water, and introduced into Russell's absorption apparatus; curve I shews no apparent inhibition of CO_2 formation, but a further experiment carried out by the aspiration method (p. 57), demonstrates that, although complete sterility is not obtained by the use of thymol under these conditions, it produces very decided inhibition. Table VI shews amounts of CO_2 as measured by the aspiration method.

method.

It is interesting to note that an erroneous interpretation might be put upon the result of the experiment in the Russell apparatus, the coincidence of the cessation of the rise of mercury in the case of the live soil and the soil plus thymol being naturally attributed to the failure of the limited oxygen supply, whereas it will be seen by comparison with the aspiration experiment that this cessation in the case of the thymol-treated soil is, at any rate in part, due to the inhibitory action of the disinfectant.

TABLE VI.

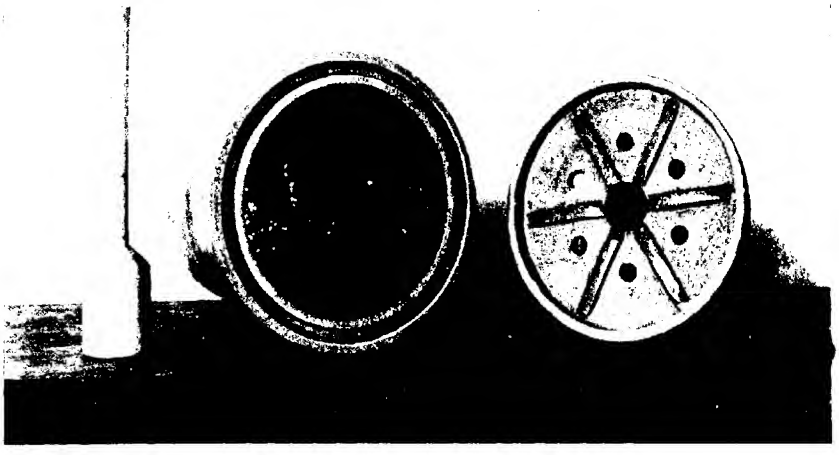
Unsterilised.		Thymol—treated.	
Mgms. of CO_2 per diem.	Total.	Mgms. of CO_2 per diem.	Total.
8·4	8·4	4·1	4·1
18·7	27·1	2·3	6·4
18·7	45·8	2·3	8·7
32·0	77·8	1·5	10·2
46·1	123·9	0·6	10·8
37·55	161·45	0·4	11·2
49·0	210·45	0·95	12·15
50·9	261·35	1·7	13·85
23·6	284·95	5·7	19·55
38·2	323·15	12·6	32·15
43·4	366·55	8·0	40·15
31·6	398·15	9·75	49·9
19·0	417·15	9·8	59·7
11·5	428·65	9·5	69·2
11·05	439·7	11·8	81·0
11·0	450·7	7·0	88·0

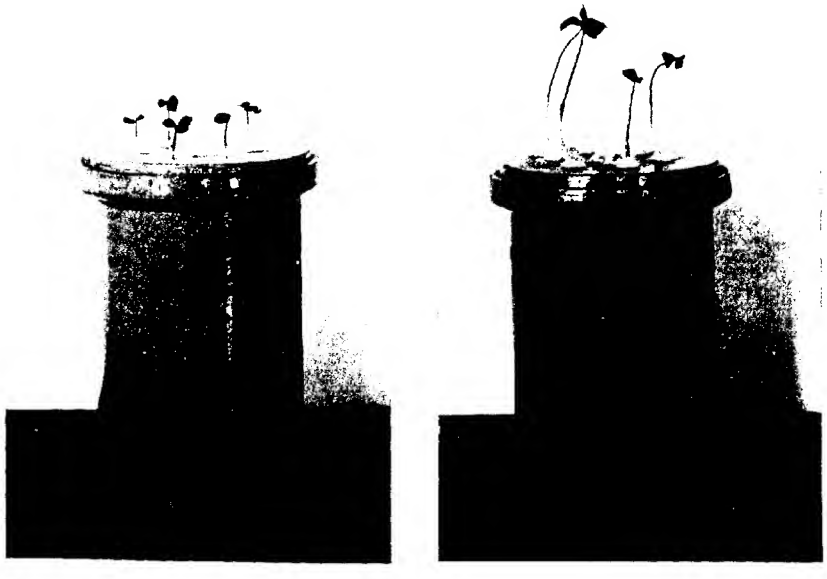
From these results the limited effectiveness of thymol, as an inhibitor of bacterial action in soil, appears to be clearly indicated. Very probably many soil organisms are comparatively unaffected by it, just as some species can develop and function in peptone broth to which toluene has been added, whilst this still

smells strongly of the latter; I have observed the same comparative indifference to chloroform on the part of many organisms, and have found that the use of this antiseptic does not by any means ensure the cessation of bacterial activity in solutions, unless precautions are taken to prevent evaporation, as bacterial action will recommence whilst the liquid is still smelling strongly of the antiseptic. I find that the addition of toluene to a mixed culture of Pusa soil organisms in peptone broth results in the eventual predominance of organisms such as C. butyricum, controls without toluene shewing formation of ammonia, indol, and skatol but no butyric odour. This was also the case with soil treated with toluene and inoculated into Mannite solution, and there is evidently a very marked selective action of such poisons, upon mixed cultures of organisms such as occur in soils, although it is by no means clear that this merely implies survival of spore-formers only.

For the purpose of pot cultures in sterile soil I have found it convenient to use pots of special design as shewn in photograph (Plate IV). These pots are of glazed earthenware, with lids fitting into an annular groove surrounding the upper edge of the pot, and provided with a porous cylinder through which the water-supply is introduced. The soil in the pot is sterilized thrice in the autoclave at 130°C., the top layer consisting of 1 to 2 inches of burnt sand, with the intention of avoiding contact during the earliest stages of growth between the germinating seed and any toxic substances which might be produced in the soil by heating, and interfere with germination and development of the radicle. The openings in the lid are occupied by short glass tubes packed round and plugged with cotton-wool; the seed previously sterilized with formaldehyde under reduced pressure, is dropped down the tubes and, after replacing the plug, is worked into the sand by movements of the glass tube. When the young plant has sprouted and grown up in the tube above the level of the cover of the pot, a collar of fresh cotton-wool is prepared and singed, the tube rapidly withdrawn and the collar put in position round the stem of the seedling; this

PLATE IV.





CULTURE POTS.

last operation must be carried out in the inoculating room. It is best to fill the pots quite up to the cover. The cover may be luted to the pot, but I have not found this necessary in practice.

Nitrification.

The work of Stevens and Withers on Nitrification in soils and in solutions (Cent. f. Bakt. 2nd Abt. Bd. XXIII-10-13) having clearly demonstrated not only the impossibility of determining the relative nitrifying powers of different soils by inoculation into Omélianský solution, but also the fact that some soils nitrify better when used as inocula in such solution than when the live soil itself is used as the medium, whilst others shew the reverse tendency, a series of experiments was undertaken with a view to determining whether Indian soils exhibited similar characteristics. The first point noted in these experiments was the fact that magnesium carbonate appeared to exercise an inhibitory influence on nitrification in Omélianský solution, and accordingly this action was investigated in a series of preliminary experiments. As will be seen from the results of this investigation the controlling factor was found to be the concentration of the solution with regard to ammonia, the amount of which liberated by the action of the magnesium carbonate and present in the solution, was sufficient to inhibit nitrification when the Omélianský medium was used at full strength, although no such inhibitory action appears to occur when $CaCO_3$ is used, even under this latter condition.

Effect of Magnesium Carbonate on Nitrification in Omélianský solution.

The composition of the Omélianský solution used was as follows :—

Ammon. Sulphate	...	...	...	... 2·0 gms.
Sod. Chloride	...	...	...	... 2·0 „
Pot. Hyd. Phosphate	...	...	...	... 1·0 gm.
Mag. Sulphate	...	...	...	... 0·5 „
Ferrous Sulphate	...	...	...	... 0·4 „
Water Distilled	...	...	...	1000·0 c.c.

100 c.c. lots of this solution were used in 250 c.c. Erlenmeyer flasks, and to each flask was added 0·5 gms. of either mag. carbonate or cal. carbonate in the form of a sterile milk in water. Incubated at 30° C.

It was found that no formation even of nitrites took place in this solution when seeded with soil, or soil extract, when magnesium carbonate was used, although calcium carbonate had no such inhibitory effect.

TABLE VII.

	Milligrams of Nitrogen as			
	Nitrite		Nitrate	
	After		After	
	14 days.	35 days.	14 days.	35 days.
Omél. Sol. + ·5 gms. $MgCO_3$	...	...	...	...
„ „ + ·25 „ „	...	...	...	...
„ „ + ·5 „ $CaCO_3$	2·9	6·5	·15	·3
„ „ + ·25 „ „	2·4	5·2	·12	·2

A further experiment was then carried out to determine whether the inhibitory action of the magnesium carbonate was a positive or negative one. Varying relative amounts of $CaCO_3$ and $MgCO_3$ were used in conjunction in a series of flasks, the same amount of a soil extract being used as an inoculum in each flask. Incubated at 30° C.

				Ratio
Omélianshy solution + ·5 gms.	$CaCO_3$	+ ·05 gms.	$MgCO_3$	10:1
Do.	„	+ ·055 „		9:1
Do.	„	+ ·06 „		8:1
Do.	„	+ ·07 „		7:1
Do.	„	+ ·08 „		6:1
Do.	„	+ ·10 „		5:1
Do.	„	+ ·12 „		4:1
Do.	„	+ ·17 „		3:1
Do.	„	+ ·25 „		2:1
Do.	„	+ ·50 „		1:1

Qualitative tests for nitrites and nitrates were made at intervals of a week; the flask with 10:1 ratio shewed reaction after first week; those with 9:1 and 8:1 ratios after second week, and the 7:1 after the fourth week, thus it appears that the action of magnesium carbonate under these conditions is a positive inhibitory one.

It was found that magnesium carbonate causes much greater loss of nitrogen from Omélianský solution (by formation probably of ammon. carbonate) than does calcium carbonate.

To two flasks each containing 100 c.c. Omélianský solution were added ·5 gms. $CaCO_3$ and ·5 gms. $MgCO_3$ respectively, and the ammonia remaining after 14 days incubation at 30° C. estimated by distillation with the following result :—

TABLE VIII.

Mgms.	N. as NH_3.		Loss.
	Original.	After 14 days.	Per cent.
Flask + $MgCO_3$	42·4	20·02	51·8
,, + $CaCO_3$	42·4	34·0	18·8

Warington (Jour. of Chem. Soc., 1884, 45, p. 637; 1891, 59, p. 484; Ashby (Jour. of Chem. Soc., 85, p. 1158), and Löhnis (Agri. Bacteriology, p. 723), have dealt with the concentration of nitrifying solutions, and the last named gives it as his opinion that no quantitative work can be done when using magnesium carbonate, and consequently recommends calcium carbonate in its place.

Two sets of experiments were made to further elucidate this point; in one set 0·2 per cent. ammon. sulphate was used and in the other 0·05 per cent. ammon. sulphate; with varying amounts of mag. carbonate and calcium carbonate, and in flasks to which sterilized soil extract had been added the loss of ammonia was periodically determined. The acidity or alkalinity of these solutions was determined, and in one flask the alkalinity due

to mag. carbonate was neutralized at the start with sulphuric acid, but soon reappeared. Results are given in Tables IX and X.

Separate flasks containing 50 c.c. of solution were used for each period of time in determining the loss of ammonia, the whole of their contents being distilled. Results are given in Tables XI and XII.

Loss of ammonia from flasks with dilute Omélianskỳ's solution (containing ·05 per cent. ammonium sulphate) + sterilized soil extract equivalent to ·5 gm. of (Pusa Botanical Area) soil : after different periods of time. Incubated at 30° C.—

TABLE XI.

MILLIGRAMS OF NITROGEN AS AMMONIA.

With dilute Omél. Solution.	After						Original.
	1 day.	2 days.	1 week.	2 weeks.	4 weeks.	6 weeks.	
Control	5·3	Not determined.				5·1	5·3
50 c.c. dilute Omél. Sol. + ·025 gms. $MgCO_3$ ...	5·18	4·90	3·64	2·52	1·26	·84	5·3
50 c.c. dilute Omél. Sol. + ·05 gms. $MgCO_3$	5·18	4·90	3·50	2·38	1·12	·70	5·3
50 c.c. dilute Omél. Sol. + ·10 gms. $MgCO_3$	5·04	4·76	3·36	2·38	0·98	·42	5·3
50 c.c. dilute Omél. Sol. + ·25 gms. $MgCO_3$	5·04	4·62	3·22	2·10	0·84	·14	5·3
50 c.c. dilute Omél. Sol. + ·5 gms. $MgCO_3$	4·90	4·20	2·80	1·82	0·70	*Nil.*	5·3
50 c.c. dilute Omél. Sol. + ·25 gms. $CaCO_3$	5·18	4·90	4·62	4·34	3·78	3·50	5·3
50 c.c. dilute Omél. Sol. + ·50 gms. $CaCO_3$	5·18	4·90	4·62	4·06	3·50	3·08	5·3

Loss of ammonia from flasks with strong Omélianskỳ's solution containing ·2 per cent. ammon. sulphate + sterilized soil extract equivalent to ·5 gms. of soil; after different periods of time. Incubated at 30°C.

TABLE XII.

MILLIGRAMS OF NITROGEN AS AMMONIA.

With strong Omél. Solution.	After						Original.
	1 day.	2 days.	1 week.	2 weeks.	4 weeks.	6 weeks.	
50 c.c. strong Omél. Sol. + ·025 gms. $MgCO_3$	19·74	19·32	17·08	16·80	16·66	15·54	21·5
50 c.c. strong Omél Sol. + ·05 gms. $MgCO_3$	19·74	19·32	14·28	11·90	9·52	8·76	21·5
50 c.c. strong Omél. Sol. + ·10 gms. $MgCO_3$	19·60	18·34	14·28	11·34	7·56	6·48	21·5
50 c.c. strong Omél. Sol. + ·25 gms. $MgCO_3$	19·46	18·06	14·14	9·94	4·76	3·22	21·5
50 c.c. strong Omél. Sol. + ·5 gms. $MgCO_3$	18·90	17·50	14·10	9·94	4·20	2·94	21·5
50 c.c. strong Omél. Sol. + ·25 gms. $CaCO_3$	20·20	19·60	18·90	16·52	15·12	14·00	21·5
50 c.c. strong Omél. Sol. + ·5 gms. $CaCO_3$	20·20	19·60	18·62	15·96	14·28	12·60	21·5
Control	21·5	Not determined.				21·5	21·5

The loss of nitrogen under these conditions has already been shewn by Ashby (Jour. of Agri. Sci., January 1907), whose experiments were carried out at the same temperature (30°C.). In India they have a greater significance owing to the fact that the temperature of the cultivated soil is at least as high as this for considerable periods. Ashby considers that this loss is due to the formation and volatilization of ammonium carbonate, and points out the correlation existing between the greater loss due to magnesium carbonate and the earlier appearance of nitrates when this salt is used in place of calcium carbonate; as the above experiments shew, under certain conditions (higher concentration of ammonium salt) the formation of ammonium carbonate under the action of magnesium carbonate is sufficiently rapid to produce inhibition of nitrification. The much greater solubility of the magnesium salt as compared with that of calcium carbonate would account for this radical difference to some extent.

An experiment in which the rate of nitrification in Oméliansky solution was measured, with the addition of sufficient sterile sand to form a slope emerging from the solution, demonstrates the effect of this method of aeration upon the nitrate formation in such a solution.

NITRIFICATION IN OMELIANSKY SOLUTION WITH SAND SLOPE.

Pusa Kitchen garden soil; one grm. in 100 c.c. (dilute) Oméliansky solution; 0·5 gms. $CaCO_3$ added.

TABLE XIII.

NITROGEN IN PARTS PER MILLION.

	AFTER					
	8 days.		10 days.		17 days.	
	Nitrite.	Nitrate.	Nitrite.	Nitrate.	Nitrite.	Nitrate.
K 1 (sand slope) ...	8·0	1·25	21·74	1·30	47	7·0
K 2 (sand slope) ...	7·0	1·50	23·63	1·75	46	9·0
K 3 (control). No sand ...	10·41	1·55	20·0	1·60	54	6·0

	AFTER					
	22 days.		28 days.		42 days.	
	Nitrite.	Nitrate.	Nitrite.	Nitrate.	Nitrite.	Nitrate.
K 1 (sand slope)	70	45·7	60	90·2	*Nil*	137·7
K 2 (sand slope)	66	50 0	50	96·4	*Nil*	162·0
K 3 (control). No sand ...	86	24·1	44	75·8	25	125·0

NITRIFICATION IN SOIL MEDIA.

Two sets of experiments were carried out to determine the optimum moisture content of Pusa soils as media for nitrification. In the first series, in which nitrogen was added in the form of rape cake, nitrification proceeded with most vigour in the soil containing the lowest amount of moisture, namely, $\frac{1}{4}$ saturation; this was most marked in the vegetable garden soil, although its

higher percentage of humus (5·7% as compared with 3·01%) raised its saturation capacity to 48% of the soil weight, whilst the other sample from the Botanical area, which lost 3·01% on ignition, was saturated with 40% of water by weight.

In the second series nitrogen was supplied in the form of ammonium sulphate in Omélianský solution which was added to the soil; additional tests were made of the relative amounts of nitrification in Omélianský solution of varying concentration, and with variations in aeration secured by using differing amounts of solution and sand slopes.

Two Pusa soils were used in this experiment.

A.—From the Botanical area to which 30% sand was added: saturation = 40% by weight.

B.—From vegetable garden: saturation = 48%. Both were air-dried and sifted through 3 mm. sieve.

400 gm. lots were placed in 48 ozs. sterilized bottles wide-mouthed and cotton-wool plugged. Six bottles of each soil were taken and water added to make three different saturations in duplicate. All bottles were then autoclaved at 130°C. for ½ hour on two successive days, and the loss of water made up to prescribed saturation, making allowance for the subsequent addition of 10 c.c. of water extract of soil as inoculum. 240 mgms. of nitrogen in the form of rape cake were then added to each bottle, and inoculation was carried out with 10 c.c. soil extract equivalent to 5 gms. soil (made by shaking 100 gms. soil in 200 c.c. water and allowing to settle). No lime was added as these soils contain over 30%.

Loss of water during incubation was made up every week (about 3 c.c. per week).

NITRIFICATION IN SOIL MEDIUM.

Series I. 240 mgm. Nitrogen added as Oilcake.

TABLE XIV.

Nitrogen Recovered.

	Parts per Million.				
	—	NH_3.	Nitrite N.	Nitrate N.	After
Pusa soil (A) (original)	...	*Nil.*	4·2	·1	2 weeks.
Pusa soil ¼ sat. (10% water)	600	126	·2	·9	
Pusa soil ½ sat. (20% water)	600	98·8	·5	·3	
Pusa soil ¾ sat. (26% water)	600	63	·2	·2	
Pusa soil (A) ¼ sat. (10% water)	600	136	3·7	3·7	4 weeks.
Pusa soil ½ sat. (20% water)	600	126	2·9	1·9	
Pusa soil ¾ sat. (26% water)	600	84	2·0	1·9	
Pusa soil (B) (original)	...	42	·7	·3	2 weeks.
Pusa soil ¼ sat. (12% water)	600	206	·6	75·0	
Pusa soil ½ sat. (24% water)	600	183	·2	12·0	
Pusa soil ¾ sat. (32% water) ..	600	105	·1	3·0	
Pusa soil B ¼ sat. (12% water)	600	210	3·7	102·5	4 weeks.
Pusa soil ½ sat. (24% water)	600	157	42·0	46·0	
Pusa soil ¾ sat. (32% water)	600	157	21·0	16·0	

Series II. Nitrogen added as Omélianski solution.

Method of adding Omélianski's solution to bottles for nitrification in soil as medium was as follows :—

A concentrated Omélianski's solution No. I was prepared of the following composition :—

26·4 gms. $(NH_4)_2SO_4$.
26·4 gms. NaCl.
13·2 gms. K_2HPO_4.
6·6 gms $Mg\ SO_4$.
5·28 gms. $Fe\ SO_4$.
In 700 c.c. distilled water.

30 c.c. of this solution contain 240 mgm. of nitrogen as ammonium sulphate. 30 c.c. of this solution were added to a measuring cylinder and the requisite amounts of water added to produce the soil saturation desired. The whole contents of the measuring cylinder were then added to the bottle containing the soil.

Inoculum for Soil Media.

100 gms. of soil made up to 200 c.c. as an emulsion in water; 10 c.c. of this, equivalent to 5 gm. soil, was used as inoculum.

Varying concentration of Omél. solution.

No. 10—30 c.c. of this solution and 10 c.c. water; this corresponds to the least quantity of water added to soil medium.

No. 11—30 c.c. of the solution and 90 c.c. water; corresponds to the highest quantity of water added to soil medium.

No. 12—30 c.c. of solution and 540 c.c. water; this is the ordinary concentration of undiluted Oméliansky's solution.

Inoculum for Omél. solution

5 gms. of soil direct.

No $CaCO_3$ was added as there is sufficient (about 30% $CaCO_3$) in the soil.

The following were prepared for comparison :—

No. 13—100c.c. strong Omél. sol. ... No lime.
1 gram soil as inoculum.

No. 14—100c.c. ,, ,, ... ·5 gram $CaCO_3$.
1 gram soil as inoculum.

No. 15—50c.c. ,, ,, ... ·25 gram $CaCO_3$.
·5 gram soil as inoculum.

No. 16—50c.c. ,, ,, ... In sand slope.
·25 gram $CaCO_3$.

No. 17—50c.c. dilute Omél's sol. ... ·25 gram $CaCO_3$.
·5 gram soil as inoculum.

No. 18—50c.c. ,, ,, ... ·25 gram $CaCO_3$.
·5 gram soil as inoculum.

NITRIFICATION IN SOIL MEDIUM.

SERIES II. 240 MILLIGRAMS NITROGEN ADDED AS AMMONIUM SULPHATE IN OMÉLIANSKY'S SOLUTION.—Period 30 days.

TABLE XV.

	N. added. Parts per million.	PARTS PER MILLION OF N. RECOVERED.				% of added nitrogen recovered as nitrate.
		Total.	NH_3	Nitrite.	Nitrate.	
1 Pusa soil (A) original	(0·039%)	...	*Nil*	1·0	7·5	...
2 ,, ,, ¼ sat.	600	87	82·0	0·5	5·2	0·87
3 ,, ,, ½ ,, ...	600	76	58·7	4·2	13·0	2·1
4 ,, ,, ¾ ,, ...	600	53	42·0	5·0	1·0	1·0
5 ,, ,, (B) original	(0·104%)	...	*Nil*	1·5	75·0	...
6 ,, ,, ¼ sat.	600	202	149·8	*Nil*	53·0	8·9
7 ,, ,, ½ ,, ...	600	200	166·0	3·7	30·0	5·0
8 ,, ,, ¾ ,, ...	600	179	176·2	0·8	1·7	0·3
9 Sand culture (saturated) 400 gms. + 130 c.c. Omél. solution ...	660	289	227·0	12·0	50·0	8·3
10 Omél. sol. 40 c.c.	6000	5810	5720·0	60·0	30·0	0·5
11 ,, ,, 120 c.c.	2000	1919	1902·5	5·0	11·7	0·6
12 ,, ,, 570 c.c.	420	388	381·0	3·1	4·2	1·0
13 ,, ,, 100 c.c.	420	417	370·0	5·0	42·0	10·0
14 ,, ,, 100 c.c. + ·5 gm. $CaCO_3$	420	...	...	4·0	62·0	14·9
15 ,, ,, 50 c.c. + ·25 gm. $CaCO_3$	420		...	60·0	80·0	19·0
16 ,, ,, 50 c.c. in sand slope ·25 gm. $CaCO_3$ ...	420	...	...	40·(	332·0	79·0
17 ,, ,, 50 c.c. + ·25 gm. $CaCO_3$	105	...		*Nil*	64·0	61·5
18 ,, ,, 50 c.c. sand slope + ·25 gm. $CaCO_3$ ...	105		...	*Nil*	90·0	86·5

Very marked differences are induced by the variation in concentration of the ammonium salt, as would be expected from the results obtained with magnesium carbonate previously described. Still greater differences are observable as the result

of the variation in the physical conditions produced by altering the volume of the liquid and breaking the surface with the sand slope. The greatest nitrate formation occurs (No. 18) where combination of low ammonia content with large air contact is provided.

It was thought that at the comparatively high temperature, 30° C., at which these experiments were conducted, sufficiently conclusive results would be obtained after one month's incubation; it is now apparent, however, that in many, if not most cases, a period of about 28-30 days is required, especially in soil media, for full activity of the nitrifying organisms to be attained; judging from numerous results obtained subsequently in connection with biological analysis of tea garden soils, this period is required for the multiplication of the organisms concerned in the various stages of nitrification up to the limit set by the conditions obtaining in the medium; the previous treatment of the soil sample generally involving desiccation, no doubt alters the composition of the bacterial complex, and, probably by destroying the accumulated bacterio-toxins, produces conditions resulting on addition of water in great multiplication of ammonifiers, which may not only interfere with the accumulation of nitrate, but may even tend to inhibit its production, raising the concentration of ammonia in the soil water above the point at which interference with nitrification begins. Ashby has shewn (Jour. of Agri. Science, Vol. II, Part I, p. 62), that nitrifiers may be gradually accustomed to work in concentrations of ammonia higher than the normal limit for their action, and we may suppose such acclimatization to take place in soil under the above conditions, the period of time above mentioned, during which in some soil media nitrification appears to be in abeyance, being required for the gradual readjustment of balance necessary for its normal continuance. A period of 48 days appears sufficient to obtain results from which reliable conclusions may be drawn as to the relative nitrifying capacity of the average Indian soils, or as to the optimal conditions under which nitrification will occur in them.

An experiment was made to determine to what extent the nitrifying power or capacity of a soil might be altered by different manurial treatment in the field; the soils were taken from the Standard Kharif plots of the Experimental Farm of the Agricultural College at Cawnpore, which had had the following treatment :—

K_1 Cattle manure ... 50 lbs. N. per acre.
K_5 Saltpetre ... 25 „ N. „
K_9 Poudrette.
K_{13} Unmanured (since 1885)

Three samples were taken from each plot at depths of 6, 12 and 18 inches respectively. Inoculation in dilute Oméliansky solution 100 c.c. + 0·2 grm. $CaCO_3$.

Incubated at 20°C. for 32 days.

Nitrogen added as ammon. sulphate = 106 parts per million.

NITRIFICATION IN OMÉLIANSKY SOLUTION.

TABLE XVI.

PARTS PER MILLION.

Sample.	Nitrite.	Nitrate.	% Nitrified.
K 6 inch	11·0	2·0	
K 12 „	23·0 } 34·8	6·6 } 10·5	14·2%
K 18 „	0·8	2·5	
K_5 6 „	21·0	3·0	
K_5 12 „	9·2 } 30·6	1·0 } 4·0	10·9%
K_5 18 „	0·4	*Nil*	
K_9 6 „	15·0	5·7	
K_9 12 „	12·5 } 35·5	3·1 } 12·3	15·1%
K_9 18 „	8·0	3·5	
K_{13} 6 „	9·6	2·3	
K_{13} 12 „	*Nil* } 10·7	*Nil* } 2·3	4·9%
K_{13} 18 „	1·1	*Nil*	

The same soils were used as soil media for nitrification; in this case, however, samples from the three depths were mixed in equal proportion and used for the experiment. Water was added to make $\frac{1}{4}$ saturation (15 c.c. water—100 gms. soil, except in the case of K_9 where 75 gms. soil—11 c.c. water were used).

The samples of 100 gms. were sterilized in the autoclave at 130°C. for ½ hour on three successive days.

One 100 gm. sample of Pusa vegetable garden soil was similarly treated for comparison.

To each sample, before sterilizing, was added 60 mgm. N. as ammon. sulphate. The soils were inoculated with 7 c.c. water extract of live soil (Pusa vegetable garden) made by shaking 100 gram soil with 200 c.c. water :—

Incubated at 20°C. for 34 days.

Initial nitrate present was determined and found to be as follows :—

K_1 5·3 parts Nitrate per million.
K_5 0·8 „ „ „ „
K_9 Not determined owing to shortness of sample.
K_{13} 0·36 „ „ „ „
Pusa soil 15·4 „ „ „ „

Organic Nitrogen was determined
K_1 0·044 per cent.
K_5 0·041 „ „
K_9 0·010 „ „
K_{13} 0·044 „ „

The comparatively low nitrogen content of the manured soils was no doubt due to the large proportion of lower soil stratum included in the samples which were, as stated above, taken down to 18 inches.

Nitrogen added as ammon. sulphate = 600 parts per million.

TABLE XVII.

PARTS PER MILLION.

Sample.	Nitrate.	Nitrite.	Ammoniacal Nitrogen.
K_1	54	·02	1·3
K_5	28	trace	1·8
K_9	54	„	0·66
K_{13}	35	„	0·74
Pusa soil	69	„	1·4

It is interesting to note in the case of these soils that sterilization in the autoclave at 130°C. on three successive days has not resulted in the production of toxins sufficient to inhibit nitrification. It is possible that toxins produced under the conditions described by Russell and Hutchinson (Journal of Agri. Science, Vol. III, Part 2) would be destroyed or dissipated by the two subsequent heatings in the autoclave; further reference is made to this point under the head of "Weathering" (p. 60).

It will be seen that the actual amount of nitrate formed, although much greater in soil medium than in solution, places these soils in the same relative positions with regard to their nitrifying power, whether this is determined by inoculation into Oméliansky solution, or in soil. It is difficult to account for the large amounts of nitrite present in the solutions, in the first foot samples, after 30 days incubation, as compared with its complete absence in the soil media, but it appears on examination of the results detailed in the previous experiment (effect of magnesium carbonate on nitrification) that this high nitrite ratio is a characteristic of low aeration, such as obtains in solutions, and is much less marked where either sand culture or sand slopes are utilized, and in the case of soil media where the lower saturations are used. It is probable that the comparatively rapid development in solutions of numerous bacteria capable of reducing nitrates to nitrites (Chester 15th Ann. Rep. Delaware Agri. Coll., 1903) may account in great degree for this observed fact.

There can be no doubt that the use of soil as a medium for measuring nitrification must give results of greater value to the agriculturist than those obtained in solutions. The estimation of relative nitrifying power in different soils, however, cannot have the same agricultural value in our present state of knowledge, as the determination of the optimal conditions of moisture, aeration, temperature, humus and mineral content, which conditions it is the business of the agriculturist to bring under control as much as possible; such determinations can only be

made in the soil itself, and it is the fact that it is possible to do this in the laboratory in such a way as to obtain data which can be correlated with field conditions, which gives perhaps its greatest value to soil bacteriology. In the case of soil chemistry there is no intermediate step between laboratory analysis on the one hand and the crop on the other. Biological analysis of a soil, however, after determination of the character and functions of the organisms present, leads naturally to further work in the laboratory, by means of which it is possible to determine under what conditions in the field the soil complex may most satisfactorily play its part in the provision of plant food. This second stage in dealing with a soil sample, includes many methods of investigation, but these may be considered as separate from the primary one of analysis by plating and culture, and dependent more upon quantitative estimation of the results of bacterial activity than upon purely bacteriological technique. I wish to suggest that this second stage should form a common ground of operations for the soil chemist, physicist, and bacteriologist; the valuable work of Lipman, Brown and Owen (Cent. f. Bakt. 2nd Abt. 31 Bd.—No. 1/4—1911) on the determination of the relative availability of nitrogenous manures by biological laboratory methods, is an example of such work, and suggests many similar lines of enquiry closely connecting laboratory investigations and field practice. I hope to be able to formulate a scheme of methods requiring no special training in bacteriological technique, although involving a sound knowledge of soil analysis, such as could be adopted in the various Agricultural Colleges throughout India.

When moisture and temperature conditions in soil become suddenly favourable to bacterial growth and activity, as happens normally in the plains of India, when the first rains moisten the soil after the long drought of winter, a very vigorous multiplication of soil organisms takes place. This has been shewn in the case of the Cawnpore soils referred to above, and it seems very probable that many of the bacteria in the soil will take their nitrogen from nitrate should this be present in sufficient

quantity, bringing about an apparent loss of nitrogen, which might be confused with denitrification proper. Soil borings made at Pusa have shewn (Memoirs of the Dept. of Agr. in India, Chem. Series, Vol. II, No. 2, "Records of Drainage in India"—J. W. Leather, 1912) an apparent downward movement of nitrates in the soil following their formation in the upper layers. Plates made from borings in this soil show the presence of bacteria down to a depth of nine feet, the lowest level reached by the bore, the numbers of which increased rapidly with access of moisture, so that the gradual penetration of rain-water would coincide with greatly increased bacterial activity. In order to gain some idea of the effect of such increase upon the nitrate present the following experiment was carried out.

Reduction of Nitrate in Soil.

In this experiment Pusa soil from the vegetable garden was used; analysis showed the nitrogen content of this soil to be as follows:—

Organic nitrogen	852·0	parts per million
Ammoniacal nitrogen	4·2	„ „
Nitrate nitrogen	1·8	„ „

The soil contained 10% moisture.

Potassium nitrate equivalent to 0·1% of soil weight was added to the soil, which was placed in glazed jars and kept at 30°C. for 8 days.

Varying amounts of water were added up to 10% – 15% and 30% of soil weight, control pots containing no added nitrate were included; each variation was duplicated. Twelve pots were used as follows:—

Water ...	10%	– 15%	– 30%		
Pots	a	d	g,	j	Nitrate nitrogen added.
	b	e	h,	k	= 0·1% soil weight.
	c	f	i,	l	control; no nitrate.

In the case of the soils containing 30% water, double sets of pots were used, as owing to the puddling of the soil, it was not feasible to draw proper samples for the two determinations made

after 4 days and 8 days respectively, it being necessary to use the whole of the soil in each pot for this purpose.

The loss of water was made up periodically by introducing the requisite quantity through porous cylinders standing in the pots. Ammonia was determined by distillation with magnesia, and nitrate by phenol-sulphonic acid method.

TABLE XVIII.

NITRATE REDUCTION :—Period 4 days.

Jar.	Percentage of water.	MGMS. OF ORGANIC NITROGEN PER KILO. OF DRY SOIL.			MGMS. OF NITRATE NITROGEN PER KILO. OF DRY SOIL.	
		Before.	After.	Increase or decrease.	Increase or decrease.	Before.	After.
a	10	852	1030	+ 178	+ 48·2	1001·8	1050
b	10	852	1015	+ 163	− 71·8	1001·8	930
c	10	852	831	− 21	− 7·8	1·8	9·6
d	15	852	996	+ 144	− 1·8	1001·8	1000
e	15	852	1013	+ 161	− 201·8	1001·8	800
f	15	852	800	− 52	+ 8·2	1·8	10
g	30	852	834	− 18	− 441·8	967·8	526
j	30	852	951	+ 99	− 487·8	967·8	480
i	30	852	816·5	− 35·5	− 0·6	1·8	1·2

TABLE XIX.

NITRATE REDUCTION :—Period 8 days.

Jar No.	Percentage of water.	MGMS. PER KILO. OF DRY SOIL. ORGANIC NITROGEN.			MGMS. PER KILO. OF DRY SOIL. NITRATE NITROGEN.		
		Before.	After.	Increase or decrease.	Increase or decrease.	Before.	After.
a.	10	852·0	895·0	+ 43·0	− 281·8	1001·8	720
b.	10	852·0	978·0	+ 126·0	− 121·8	1001·8	880
c.	10	852·0	840·0	− 12·0	+ 7·8	1·8	9·6
d.	15	852·0	953·0	+ 101·0	− 601·8	1001·8	400
e.	15	852·0	904·0	+ 52·0	− 501·8	1001·8	500
f.	15	852·0	835·0	− 17·0	+ 6·7	1·8	8·5
h.	30	852·0	925·0	+ 73·0	− 692·8	967·8	275
k.	30	852·0	820·0	− 32·0	− 692·8	967·8	275
l.	30	852·0	801·0	− 51·0	+ 0·8	1·8	2·6

From this experiment it will be seen that when the percentage of nitrate present reaches a certain point, in this case 0·1 per cent., and when the moisture content is near the optimum for bacterial growth and activity, a very considerable reconversion of nitric nitrogen into organic nitrogen may take place; even in such a short period as 8 days the amount of nitrogen thus converted corresponds, in the case of the soil used in this experiment, containing about 10 per cent. moisture to an amount equivalent to 138·5 lbs. nitrogen per acre in the first 9 inches. It appears probable, therefore, that a considerable proportion of nitrate artificially added to soils as manure is used up by bacteria, and, if it ever reaches the crop, does so only after being again nitrified. Chester (loc. cit.) has shewn that many common soil bacteria, such as B. subtilis, B. mycoides, B. cereus and others, reduce nitrates to nitrites, and this has been found to be the case with many organisms in Pusa soil, from which it follows that the apparent loss of nitrates in soils is due to a combination of causes of which denitrification, that is the reduction of nitrates to nitrogen gas, is not necessarily one in every case, nor is it correct to assume that translocation has taken place on account of the observation of lowering in amount of nitrate in one soil stratum with corresponding increase in another. Experience has shewn that the optimum amount of moisture for nitrification in Pusa soils is low, so that rapid nitrification will probably set in with the advent of the first rains, whilst the soil is still comparatively dry; later the rise of moisture content will check the rate of nitrification whilst that of multiplication of other soil organisms may continue comparatively unimpaired, with a corresponding reduction of the nitrate formed. This action may be supposed to accompany the gradual penetration of the rain-water to lower levels of the soil, thus accounting to some extent for the apparent gradual movement of nitrates downwards. It may be objected that the concentration of nitrate in this experiment (0·1 per cent.) is very much higher than any actually occurring in field soils, but the concentration determined by analysis assumes the diffusion of the nitrate throughout the volume of

soil in which it is found, whereas it must be remembered that in dealing with bacterial action we have to consider the condition of concentration in the immediate neighbourhood of such particles of nitrogenous organic matter as are undergoing the various chemical changes incidental to nitrification, and it may quite well be that sufficient concentration occurs at such points to produce conditions entirely different from those which would obtain if regular diffusion were going on without such local disturbances. So far as chemical action due to biologic activity in soils is concerned, it seems necessary to assume that each particle of organic matter undergoing decomposition by bacterial agency, constitutes a centre from which radiate gradients of concentration of the various soluble compounds resulting from such decomposition. Not only would the physiological functions of the bacteria present be modified by such variations in concentration, but it is probable that chemotaxis would largely determine their position along such radii. The complexity of the problem makes it impossible in our present state of knowledge to follow this line of argument further, but it seems necessary to remember that, in considering chemical changes in soil due to biologic activity, we are dealing with discrete particles, and not with the interaction of substances in solution and equally dispersed throughout the mass of soil under examination; that such localized differences of action can occur, we have evidence of in the case of those anaerobes, such as Clostridium Pasteurianum, which can carry on their normal physiological functions under aerobic conditions, in the presence of aerobic bacteria whose vital processes suffice to lower the oxygen tension in their immediate vicinity.

In another experiment designed to test the nitrifying capacity and efficiency of a tea garden soil, from Allynugger in Sylhet, this reducing action was clearly shewn. In this case 400 gm. lots of soil were mixed with mustard cake containing 240 mgm. nitrogen; and to these were added, in one set, lime and in another magnesium carbonate, equivalent to one per cent. of the soil weight; a third set had no addition; water was added

to make $\frac{1}{4}$ saturation in one series and $\frac{3}{8}$ saturation in the other; nitrates and nitrites were estimated after 14 days and 28 days, separate bottles and samples being used for each determination. The results are given in Table XX.

ALLYNUGGER TEA SOIL.

400 gms. soil + 240 mgm. N. as oil cake.

TABLE XX.

MILLIGRAMS OF NITROGEN.

After 14 days.

Saturation.	Soil.		Soil + lime.		Soil + $MgCO_3$	
	Nitrate.	Nitrite.	Nitrate.	Nitrite.	Nitrate.	Nitrite.
$\frac{1}{4}$	4·4	*Nil.*	13·2	*Nil.*	5·2	0·6
$\frac{3}{8}$	5·2	*Nil.*	5·2	*Nil.*	7·4	0·2

After 28 days.

Saturation.	Soil.		Soil + lime.		Soil + $MgCO_3$	
	Nitrate.	Nitrite.	Nitrate.	Nitrite.	Nitrate.	Nitrite.
$\frac{1}{4}$	5·2	0·8	5·2	2·2	3·0	3·2
$\frac{3}{8}$	7·4	0·5	7·4	2·0	6·7	4·2

It will be seen from these figures that a considerable formation of nitrite has taken place between the 14th and 28th days of incubation, with an apparent reduction of nitrate, which is very marked in the case of the soil + $MgCO_3$, and still more so in that with lime. I do not propose to discuss here the remarkable differences caused by varying saturations, as this point is being further investigated, but it is obvious that great differences will arise as a consequence of the varying concentration of salts in the soil water, and the effect of such variations upon the physiological functions of the micro-organisms of the soil.

The depressing effect of excess of magnesium carbonate upon the amount of nitrate is very marked in the lower saturation, and is followed, after 28 days incubation, by a correspondingly high proportion of nitrites.

The reduction of nitrate which takes place in water-logged soil is a well recognized fact, but it may be of interest to cite an experiment, shewing the rapidity with which such action may take place, and suggesting that it may be responsible for the destruction of the major portion of nitrate present in a soil at any one time, under conditions which might be considered normal, and consequently harmless in this respect.

TABLE XXI.

NITRATE REDUCTION IN WATER SATURATED SOIL.

	No.	Nitrogen as Nitrate.	Nitrogen as Nitrite.	Total N.
June 19th	29	17·45	4·2	21·65
	30	11·15	2·4	16·55
„ 20th	29	8·05	13·2	21·25
	30	7·30	8·4	15·7

Nos. 29 and 30 were duplicates of same soil, but No. 30 had an addition of lime, No. 29 being untreated.

In this case saturation for 24 hours resulted in the reduction of 50 per cent. of the nitrate present, this latter having been formed naturally in the soil from oilcake added 4 weeks previously. It is easy to realize that heavy rain would probably produce in the field an exactly similar result under conditions in which the soil might readily be supposed to be free from such reduction, so that, during the rains in India, it is improbable that nitrification is likely to be indicated by accumulation of nitrate, and inferences as to its formation and distribution based on periodic sampling would depend for their value upon such samples being drawn with sufficient frequency to avoid error due to variation resulting from the causes above indicated.

In considering the possibility of nitrate reduction in soil it is necessary to bear in mind the conditions under which this takes place in conjunction with the decomposition of cellulose. Van Iterson has demonstrated (Cent. f. Bakt. XI, No. 23), the formation of nitrites as an intermediate step in this process, and has shewn that anaerobic conditions are by no means necessary for the action of the specific organisms concerned. The importance of this action in connection with the biological and chemical phenomena associated with the practice of green manuring will be easily realized. Experiments made at Pusa, and dealing with the fate of plant tissues after burial in soil, details of which will form the subject of a future memoir, shew clearly the intimate relation between soil nitrate and the decomposition of organic matter. It is, then, important to realize that the reduction of nitrates generally associated with anaerobic conditions and the evolution of nitrogen gas, may take place under aerobic, or semi-aerobic, conditions, the fate of the nitrogen depending upon whether it reverts to organic combination as bacterial food, or is lost as ammonia or free gas. One other consideration arises; competition between nitrifiers and other soil organisms for the supply of nitrogen available might determine the rate of nitrate formation and might even inhibit it altogether.

The action of green farmyard manure upon soil nitrates must not be overlooked, as, although no experiments under critical conditions have been carried out in India, there can be no doubt that the enormous numbers of bacteria introduced into the soil when fresh cow manure is utilized, must exercise a very decided influence in determining whether the supply of nitrogen should exist mainly in the organic or in the oxidized condition. It has been estimated that from 5 to 20 per cent. of the dry weight of animal fæces may consist of bacterial cells, which would readily account for the diminution of the amount of nitrate which has been demonstrated to follow the use of green farmyard manure, without falling back upon the assumption of denitrification in its usual sense.

An indication has been obtained from a further series of experiments, not yet fully worked out, that soil temperature is a determining factor in deciding whether the ultimate result of bacterial action will be the formation of nitrate in any considerable quantity. Thus at 15°—20°C. ammonification and nitrification proceed concurrently in Pusa soil containing added organic matter, whereas at 28°—30°C. ammonification is so rapid that nitrification is inhibited for several weeks, owing apparently to the concentration of ammonia in the soil water; at this higher temperature ammonia is given off as gas from the soil, and it is not until much of the nitrogen of the organic matter has been lost in this way that nitrification begins.* Such loss of nitrogen will depend not only on temperature and the presence of sufficient moisture, but upon that of a comparatively large excess of organic matter, and in practice the conservation of nitrogen will depend upon a careful study of these considerations, which may be summed up as suggesting the avoidance of the conjunction of high temperatures, excess of water or of organic manures. Biological analysis of a soil will provide useful information as to the time of year at which to apply organic manures, the best kind to use, and as to the optimum amounts of manure and water required for nitrification.

It appears probable, therefore, that in Indian soils, nitrogen passes through a comparatively numerous and rapid series of combinations, no large accumulation of nitrates being likely to take place, except in very special circumstances, such as would probably allow of the formation of nitrate under optimal conditions of aeration and water-supply and its continuous removal to another soil stratum where the water-supply was insufficient to allow of excessive bacterial activity. It is probable that the saltpetre industry in India depends upon the occurrence of such conditions. So far as soil fertility is concerned, the plant has to compete for its nitrogen with such soil bacteria as are

* This range of soil temperature is that which occurs over a large part of India, and in this respect we find a decided difference between biological soil changes in this country and in N. Europe.

liable to interfere with the formation of nitrate, either by taking up proteid nitrogen which would otherwise have been nitrified, or nitrate nitrogen which would have been used as plant food. The management of soil must, therefore, include operations calculated to assist the crop in this competition, and the successful application of the science of soil bacteriology to agriculture will depend largely upon the acquisition of knowledge bearing upon this problem. A special case, but one of great importance, is the cultivation of wheat under irrigation; it is a well recognized fact, that injudicious application of water will prejudice the growth of this crop, just as unseasonable rainfall does in unirrigated tracts, in a manner which indicates that such application has reduced the supply of available nitrogenous food; Howard has shewn at Pusa how such damage may be partially remedied by application of nitrate of soda, thus confirming the view that it is due to loss of nitrogen; the smallness of the amount of water necessary to produce this prejudicial effect precludes the possibility of removal of nitrate by drainage, and it seems necessary to conclude that reduction takes place as a consequence of the stimulation of bacterial activity resulting from the addition of water to the soil. The study of the problem, *i.e.*, the optimum method of distributing the water-supply, in such a way as to stimulate the various micro-organisms whose combined activity results in nitrate formation, and yet avoid their subsequent competition with the plant for the nitrate thus produced, will afford scope for a very large amount of bacteriological investigation, but its importance wherever irrigation is practised would fully justify the expenditure of such time and labour as may be required for its elucidation.

The problem itself may be briefly summarized as follows; empiricism has determined that application of water at the wrong time is capable of doing great damage to a crop, which damage is probably due to interference with the supply of nitrogen. This supply depends upon the intervention of soil bacteria, beginning with those whose function is to break down

nitrogenous organic matter as a preliminary to the formation of nitrate by nitrifiers; it is found that competition for nitrate may occur between soil bacteria and growing plants, and that the numbers of the former may be increased, and this competition made more severe, by injudicious application of water. The ideal to aim at appears to be, first, to have the highest number of soil bacteria breaking down organic matter in the soil and preparing it for nitrification before the *rabi* crop is sown; later, to keep down the number of soil organisms so as to avoid reduction of nitrate and competition with the growing crop. The object of the enquiry would be to determine the conditions under which such control could be obtained and how best to exercise it.

It may be noted as relevant here that the practice, known locally in the Shahabad district as "Nigar," is an example of the arrival by empirical methods at an appreciation of the value of modifying the supply of water to a soil in accordance with the nitrogen requirements of the crop growing on it. In this case the crop is rice, growing on irrigated land, and actually under water from the time of planting out from the seed bed up to the moment when the water is run off the fields; the point of growth of the crop at which this is done is decided by the cultivator, whose experience in the districts where "Nigar" is practised tells him that a better crop will be secured in this way than by allowing the water to remain until later in the season. It is pointed out later on page 54 that, according to the researches of the various workers quoted, the rice plant in the earlier stages of its growth takes up nitrogen not as nitrate but as ammonia; it has been further found, however, that fuller maturity is secured if nitrate is supplied during the later stages of growth, and herein lies the explanation of the success of the practice of "Nigar" which, by altering the anaerobic condition obtaining whilst the soil remains under water to normal aeration, permits nitrification to set in, and thus secures the supply of nitrate required for the later stages of the rice plant's growth.

"Weathering" of Indian Soils.

A remarkable increase in fertility has been shewn (Howard Memoirs, Dept. of Agri. in India, Bot. Series, Vol. III, No. 4) to follow the practice of "weathering" Indian arable soils. This operation consists in repeated ploughing of the soil during the hot dry season previous to the first rains of spring. It was suggested (Nature, Vol. LXXXII, p. 456), that this increase might be due to a partial sterilization effect following on the exposure of the soil to the heat of the sun, and the following series of experiments was undertaken with a view to obtaining some information as to the biological changes produced in the soil by such treatment. Samples of the "weathered" soil were compared with the same soil unweathered, and soils artificially weathered in the laboratory by heating were compared with these and with others which had undergone various methods of partial sterilization. It was found that the maximum temperature reached in the sun was 60°C. for the top $\frac{1}{4}$ inch, and although it could not be supposed that more than a small fraction of the whole soil under treatment would be brought in actual practice to this degree of heat, this temperature was adopted as the maximum in the artificial weathering experiments.

Weathering.

Pusa Soil. Botanical area—1st six inches. Artificial weathering was effected by exposing the soil to the heat and light of a Nernst lamp for 8 hours daily for one week, at such a distance that the maximum temperature reached on the surface was 60°C. The soil was stirred with a sterilized glass rod once every day.

Plates were made in Agar (+ 5 Fuller) with following dilution :—

10 gms. soil in 250 c.c. water.
1 c.c. in 250 c.c.
1 c c, in 10 c.c. agar.

After weathering 2 per cent. moisture remained in the soil; this corresponded with the amount found in this soil when air-dried.

Number of colonies after 48 hours incubation at 30°C.

Weathered	soil	1st inch	average	20 colonies.
„	„	2nd „	„	50 „
Unweathered	„	1st „	„	150 „

TABLE XXII.

THE KINDS OF ORGANISMS FOUND WERE

Weathered, 1st inch.	Weathered, 2nd inch	Unweathered.
B. Mycoides ...	B. Mycoides	B. Mycoides.
B. Subtilis	B. Subtilis	B. Subtilis.
B. Mesentericus ...	B. Mesentericus	B. Mesentericus.
	Blue Fluorescent colonies: spreading: motile rods.	Blue Fluorescent round colonies short motile rods. Round yellow colonies; motile rods.
		Sarcina yellow.
		„ brown.
		„ pink.
		„ white.
		Micrococcus Cand.
		* Streptothrix, white.
		* Yeasts, pink.
		„ white.
		Trichosporium.
		Cladosporium.
		Penicillium.
		* White slimy colonies.
		* Aspergillus.

* Additional plates were made on Synthetic Agar when certain other organisms (marked with an asterisk) appeared in addition.

It will be seen from Table XXII that a very marked influence upon the constitution of the soil complex is exerted by the operation of weathering, resulting in the elimination of all but the sporing forms characteristic of the subtilis group; as these forms have been shown (p. 10) to possess superior ammonifying power as a whole to those eliminated by weathering, one

is tempted to suggest that this selective action may be one of the contributory causes of the resulting fertility.

NITRIFYING POWER.

The weathered soil was seeded into Oméliansky solution, in duplicate, and incubated at 30°C.

TABLE XXIII.

NITROGEN IN PARTS PER MILLION.

	Weathered 1st Inch.		Unweathered.	
	Nitrites.	Nitrates.	Nitrites.	Nitrates.
After 7 days ...	19·2	3·5	25·0	3·0
	22·7	3·5	27·5	3·5
„ 14 „ ...	80·0	4·6	74·0	4·4
	83·3	5·6	80·0	5·5
„ 28 „ ...	82·4	60·4	80·0	61·8
	86·2	68·0	83·0	66·0
„ 42 „ ...	70·2	90·6	60·0	99·0
	71·4	96·0	56·0	104·0
„ 70 „ ...	*Nil*	208·3	*Nil*	227·2
	Nil	208·3	*Nil*	217·4

From this table it will be seen that the nitrifying power of the soil was not destroyed by weathering, nor was there any difference in the rate of nitrification. Considering the fact that only the top layer of the soil is raised above the thermal death point of the nitrifying organisms, and that reinfection of this layer is bound to occur when the next ploughing takes place, this result is naturally to be expected in the field.

The effects of various methods of partial or complete sterilization including "weathering," upon the number of colonies appearing on agar plates were shewn on p. 15. The results of such treatment upon the ammonifying and nitrifying power of the soil, upon nitrogen fixation in mannite solution, and upon protozoal content, are given in the following tables.

AMMONIFICATION IN RÉMY SOLUTION.

TABLE XXIV.

Inoculum 1 gram soil. 50 c.c. Rémy solution.	Milligrams N. as Ammonia.	
	6 hours.	24 hours.
Unsterilized soil	1·12	1·54
Ditto ,, + cake	1·40	2·10
Heated to 60°C. for three days	0·56	0·98
,, ,, 100°C. ,, three hours	0·84	1·26
5% Toluene	0·91	1·40
Heated to 100°C. for one hour on three successive days	*Nil.*	0·63
Autoclave 130°C. ½ hour on three days	*Nil.*	*Nil.*
Soil + cake ,, ,, ,, ,, ...	*Nil.*	*Nil.*
Soil heated to 150°C. hot air oven ½ hour on three days	*Nil.*	*Nil.*

Further determinations were made by inoculating into Rémy solution after storing some of the treated soils for seven weeks.

TABLE XXV.

Milligrams N. as Ammonia in 50 c.c. Rémy Solution.

	After	
	7 days.	14 days.
1. Untreated	13·49	28·00
2. Heated to 60° C.	22·01	43·40
3. Heated to 100° C. on three successive days ..	7·53	23·80
4. 5% Toluene	24·14	45·8

A further experiment was made to determine the relation, if any, between the presence of protozoa in the soils after treatment and the ammonifying power of the latter. The soils after treatment were seeded into hay infusion which was examined daily; no protozoa were found in any of the flasks until the sixth day; two types were found to occur; in some cases both were found, in others only one, but it is to be remarked that these experiments were carried on and repeated at various seasons of the year, and no protozoa were found by the above or other method between the months of November and May.

AMMONIFICATION IN RÉMY SOLUTION.

TABLE XXVI.

MILLIGRAMS OF NITROGEN AS AMMONIA IN 50 c.c.

	After 7 days.	After 14 days.	Protozoa kinds.
1. Control	21·7	28·0	Two
2. Sun, 7 days of 6 hours max. temp. 55°C. ..	20·3	37·1	One
3. Water oven 60°C. ; 7 days of 6 hours ...	19·8	36·4	None
4. Electric oven 55°C. ; 7 days of 6 hours ...	20·3	42·0	Two
5. 100°C. (Koch steamer) ; 3 hours once ...	5·6	19·6	None
6. 100°C. (Koch); one hour on two successive days	1·4	2·8	None
7. Autoclave 130°C. ; half an hour	7·0	7·7	None
8. Do. do. on two successive days ...	1·4	3·5	None
9. Hot air at 150°C. for half an hour ...	4·2	4·9	None
10. Hot air at 150°C. ; half an hour on two successive days	2·8	3·5	None
11. Thymol 2 c.c. saturated solution to 20 gms. soil	19·6	25·2	None
12. Salicylic Acid as in No. 11	21·0	27·3	Two
13. Formalin vapour 24 hours	3·5	35·5	None
14. 5% Toluene	20·3	39·2	None
15. Exposed to minimum night temperature 47° F. for 7 nights	21·0	35·7	None

1·0 mgm. ammonia was obtained on distilling 50 c.c. blank Rémy solution for half an hour.

Soil after the above varying treatment was seeded into Omélianský solution ; after 28 days small quantities of nitrates were found but only in the following :—

TABLE XXVII.

Period 28 days.	Mgms. of Nitrogen as Nitrite.	Nitrate.
No. 1. Control untreated	7·3	0·575
No. 2. Sun heat	7·7	0·625
No. 11. Thymol	7·1	0·737
No. 12. Salicylic Acid	8·0	0·525
No. 15. Low temperature	7·7	0·862

It is interesting to note that neither Thymol nor Salicylic acid have destroyed the nitrifying organisms in these soils. A trace of nitrite was found after 4 weeks in the soil heated to 60°C. This was therefore kept under observation and fresh determinations shewed the following amounts of nitrite and nitrate after 10 weeks.

TABLE XXVIII.

Period 10 weeks.	Mgms. Nitrogen in 100 c.c. Solution.	
	Nitrite.	Nitrate.
Control	8·33	1·470
Heated to 60°C.	7·14	1·125

Comparison of ammonifying power after varying treatment with survival of protozoa, as in Table XXVI, shews no coincidence between elimination of the latter and increased ammonification: thus, in No. 4 heated in the electric oven to a maximum temperature 55°C., the ammonification is increased in much the same ratio as in No. 14 treated with toluene; although in the former both kinds of protozoa survive, in the latter all are eliminated. It is of interest to note that the soil protozoa, or at any rate both of the two kinds found in Pusa soil, survive the temperature 55°C. but succumb to that of 60°C., these two points coinciding with the average maximum and the highest observed temperature respectively in the surface soil. Protozoa were first found in the wheat soils of the Botanical area in May, that is to say, after the "weathering" had been in operation for nearly the whole of its normal period; it does not seem likely, therefore, that the operation of "weathering" can depend for its effectiveness upon the removal of protozoa from the soil: on the other hand, although the temperature reached in the soil is not sufficient to destroy all the protozoa present, it is extremely probable that the state of desiccation to which the soil is brought is sufficiently high entirely to inhibit the activity of such comparatively large

organisms, whilst it has been shewn that great bacterial activity and multiplication can occur in comparatively dry soils, although this is apparently limited to certain special forms.

As above mentioned, no protozoa of any kind were found in the ordinary arable farm soils at Pusa during the period November to May, although these soils had not undergone any "weathering" process. It is, of course, quite possible that protozoa were present, but not in sufficient numbers to be discovered by the method of introducing the soil into hay infusion. It is difficult to conceive of bacterial life in such soils being seriously affected by phagocytic organisms so sparsely distributed through them.

NITROGEN FIXATION IN MANNITE SOLUTION.

One gram of soil was seeded into Mannite solution (Ashby's formula).

TABLE XXIX.

NITROGEN FIXED IN MILLIGRAMS AFTER 21 DAYS AT 30°C.

1.	Unsterilised soil	... 8·236	
2.	" +cake ...	... 8·378	*Azotobacter present.*
3.	Soil heated to 60°C. ...	... 7·668	
4.	" " " 100°C. ...	... 2·130	
5.	Soil Toluened ...	... 3·418	*Butyric acid present.*
6.	Soil heated in Koch 3 days ...	... 1·704	
7.	" " " Autoclave 3 days	... 1·704	
8.	" + cake autoclave 3 days	... 1·846	
9.	" 150°C. 3 days	... 710	
	Control Flask of mannite solution	... 710	

In Table XXIX are given the results of introducing soil variously treated into Mannite solution. It will be seen that soil heated to the maximum temperature reached in "weathering" was practically unaffected so far as its content of nitrogen fixing organisms was concerned. A noticeable feature of this experiment was the butyric fermentation characteristic of the toluene treated soil, probably due to the survival of a Clostridium ;

this is an almost invariable result, when toluene is added to liquid cultures of Pusa soils, and allowed to evaporate slowly.

Effect of "weathering" upon Physical character of soil.

Soil variously treated as above was shaken with water and the suspension allowed to settle in Nessler cylinders for 48 hours. The ratio of the depth of clear liquid to the total column is given in Table XXX.

TABLE XXX.

Effect of varying treatment upon sedimentation.

		Per cent.
1.	Control	25
2.	Sun heat 50°C.—7 days	100
3.	Water oven 60°C. 7 „	100
4.	Electric oven 60°C. 7 „	100
5.	Koch 100°C. 3 hours continuously	50
6.	Koch 100°C. 1 hour 2 successive days	100
7.	Autoclave 130°C. ½ hour once	50
8.	Autoclave 130°C. ½ hour 2 successive days	100
9.	Hot air 150°C. ½ hour once	100
10.	Hot air 150°C. ½ hour 2 successive days	100
11.	Thymol	25
12.	Salicylic acid	20
13.	Formaldehyde vapour 24 hours	100
14.	Toluene 5%	25
15.	Minimum night temperature 47° F.	30

It will be seen from the above table that heating to 50°C. in the sun discontinuously for seven days produces the same effect as 150°C. in the hot air oven for ½ hour; whereas 100°C. in the Koch for three hours continuously produces only half this effect. Discontinuous heating in the Koch as in No. 6 (one hour on two successive days) produces the maximum effect, although the total duration of the heating in this case is only two hours as compared with three in No. 5. The relation between the complex chemical changes produced by heating soils and the effect of such treatment upon the soils considered as culture

media for bacteria, will be referred to later in connection with the presence of bacterio-toxins in soil.

Reduction of Nitrates.

This was found to occur in a Dextrose-Nitrate solution, in the case of all the soils excepting those which had been completely sterilized by various treatments; to this there was one exception, namely, the soil heated to 60°C. in the water oven, in which, as in the completely sterile soils, nitrates were still found after 32 days. There is thus in this case the same line of division between the temperatures of 55°C. and 60°C. as was found to coincide with the elimination of protozoa and of nitrifying organisms

Soil variously treated as above was inoculated into the following medium to determine the effect of such treatment upon those organisms capable of reducing nitrates :—

Dextrose	...	...	...	15 grams.
Pot. Nitrate	...	...	...	3 „
Pot. Phosphate	...	...	...	1 „
Water	...	...	...	1000 c.c.

The cultures were examined every 48 hours and after 7 days nitrates were still present in all; after 9 days no nitrate was found in No. 1 (control); after 14 days no nitrate was found in No. 5 (Koch 2 hours) and after 17 days no nitrates were found in any of the flasks with the following exceptions :—

Koch : twice heated.
Autoclave—once, and twice.
Hot air 150°C. once, and twice.
Formalin vapour.
Heated to 60°C.

It is interesting to note that the maximum "weathering" temperature 60°C. apparently eliminates bacteria capable of reducing nitrates in liquid cultures, but as it has been shewn that such bacteria as B. Subtilis and B. Mycoides survive this temperature, it is difficult to reconcile this with the accepted view that the former species, in common with many other soil bacteria, actively performs such reduction.

EFFECT OF PARTIAL STERILIZATION ON ANAEROBIC ORGANISMS.

Soil variously treated was plated and incubated under anaerobic conditions.

20 gram lots of soil were introduced into sterilized Erlenmeyer flasks of 250 c.c. capacity and treated as follows :—

No. 1. Control
No. 2. Sun heat 7 days.
No. 3. 60°C. water oven 7 days of 6 hours.
No. 4. 55°C. Electric oven 7 days of 6 hours.
No. 5. Koch 100°C. ½ hour on two successive days.
No. 6. Autoclave 130°C. ½ hour " "
No. 7. Hot air oven 150°C. ½ hour " "
No. 8. Thymol 10% saturated solution.
No. 9. Formalin Vapour 24 hours.
No. 10. Toluene 5% for 3 days. Toluene then evaporated.

A water extract of these soils was made by shaking with 100 c.c. sterile water; two loops inoculated in 10 c.c. synthetic agar and plated; incubation at 30°C. under anaerobic conditions obtained by filter pump and use of alkaline pyrogallic acid. At the same time a duplicate set of plates was made and incubated in air for comparison.

TABLE XXXI.

	Colonies.	
	Anaerobic.	Aerobic.
No. 1. Control	629	5,550
No. 2. Sun heat	148	2,590
No. 3. Water oven	111	1,850
No. 4. Electric oven	185	3,060
No. 5. Koch	None.	740
No. 6. Autoclave...	None.	None.
No. 7. Hot air	None.	None.
No. 8. Thymol	481	3,330
No. 9. Formalin	None.	None.
No. 10. Toluene	296	4,440

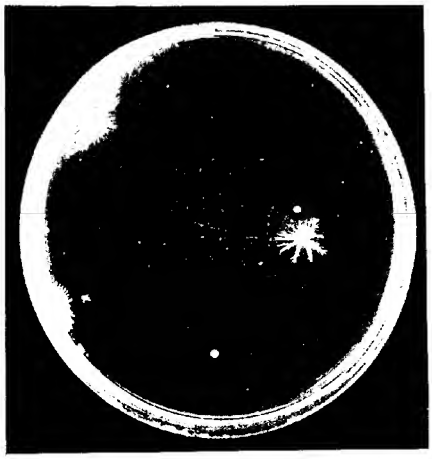
The relative reduction in the number of anaerobic and aerobic organisms effected by treatment is shewn in Table XXXI, from which it will be seen that the former suffer much more severely from the effects of partial sterilization than do the aerobes. It was found in a subsequent experiment that artificial weathering at a temperature of 55°C. after 14 days' continuous treatment, entirely eliminated all anaerobic organisms capable of developing on agar plates. The conditions during "weathering" in the field would therefore tend in course of time to eliminate the anaerobic flora, and in so far as these are associated with unfavourable acid fermentations, to improve its general condition; it would be interesting to discover whether such radical alteration in the soil complex might not, however, belong to that class of phenomena generally referred to as "disturbing the balance of nature," and it is easy to conceive of a condition in which a great reduction in the number of those anaerobes mainly responsible for the decomposition of cellulose might render a soil unfit to profit by the use of green manuring. Intensive cultivation has been shewn to result in a loss of "condition" in arable soils, and it is possible that this may be due in part to such a disturbance of equilibrium as I have suggested. It has been found by Coleman* in Mysore that aeration of paddy soils by cultivation after harvest has a decidedly prejudicial effect upon the succeeding paddy crop. In this case we have a disturbance of the conditions favourable to the preponderance of anaerobic flora, and as the rice plant depends for its nitrogen mainly upon the decomposition of organic matter under anaerobic conditions in the puddled soil, and in the earlier stages of its growth takes up this element in the form of ammonia and not as nitrate,† it is conceivable that aeration of the soil may interfere with the normal processes favourable for the growth of this plant, firstly, by the formation of nitrates which will be washed out of the soil when puddled, this

* Experiments on paddy cultivation 1909-11—Bulletin No. 2, Department of Agriculture, Mysore State.

† *Kellner*—Landwirth Versuchs—Station Vol. 30. 1884.

Kelly—Bulletin 24, Hawaii Agricultural Experiment Station, 1911.

PLATE V.



a

b

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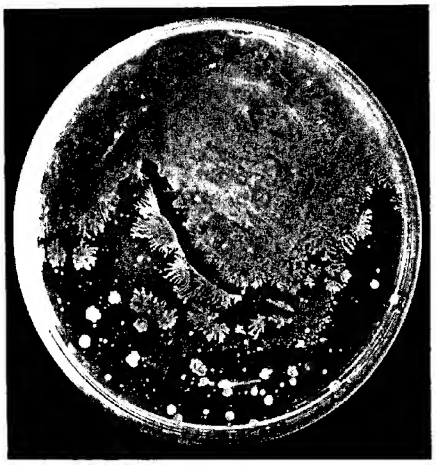


d

SOIL EXTRACT PLATES. B. PRODIGIOSUS.

(*a*) Soil extract untreated.
(*b*) ,, ,, boiled.
(*c*) Extract of Toluened soil.
(*d*) ,, Weathered soil.

loss probably being increased subsequently by denitrification, and secondly, by the reduction in the numbers of the anaerobic organisms upon which the rice plant depends for its nitrogen supply during growth after transplanting.

In addition to these observed effects a further one is produced, the bearing of which upon fertility has been pointed out by Greig Smith (Cent. f. Bakt. II Abt. Bd. 30, No. 7/12) who has shewn that water extracts of certain soils exercise an inhibitory effect upon the growth of bacteria, which can be modified by heat, by sunlight, and by exposure to air. These results are in accord with the assumption of the existence of bacterio-toxins in soil, and Greig Smith has found a quantitative relation between the fertility of certain soils and their content of toxin. I have applied this method to Pusa soils and have also tested the effect of varying treatment of the soil upon the toxic action of the water extract.

Plate V shews the results of plating pure cultures of B. Prodigiosus inoculated into soil extracts after varying treatment of the soil or of the extract. Here the varying number of colonies may be assumed to be due to the variation in the content of bacterio-toxin in the several soil extracts resulting from different treatment, so that plates shewing large numbers of colonies will result from extracts containing small amounts of toxin and *vice versâ*. It will be seen that "weathering" the soil produces a similar effect to boiling the soil extract, and there can be but little doubt that the bacterial activity of a soil is enhanced by the destruction of bacterio-toxins resulting from exposure to sunlight and air. The action of toluene is similarly pronounced, and as the whole of the soil sample treated with toluene was utilized in making the water extract, the want of toxicity in the latter cannot have been due merely to redistribution of the toxin in the soil.

The fact that such bacterio-toxins gradually lose their toxicity in water solution, and on exposure to air and light, must be taken into account in dealing with experiments involving water cultures or filtration.

In order to determine whether partial sterilization by toluene might not depend largely for its effect upon alteration either of the texture and permeability of the soil, or upon removal of some deleterious substance, probably by the solvent action of the antiseptic, an experiment was arranged in which the action of protozoa and phagocytes generally was eliminated by preliminary heating of the soil for $\frac{1}{2}$ hour in the Koch steamer. The soil, which was a tea garden soil (Doom Dooma—Assam) after this heating was divided into three lots, A, B, C, of 100 gms. each, which received the following treatment :—

A had no further treatment and served as a control.

B was treated with 5 gms. of toluene which, after being thoroughly incorporated with the soil, was allowed to evaporate for 48 hours.

C was treated with excess of toluene and kept saturated and occasionally shaken for 24 hours, after which the excess of toluene was drained off and the remainder got rid of by passing a current of air over the soil for 24 hours.

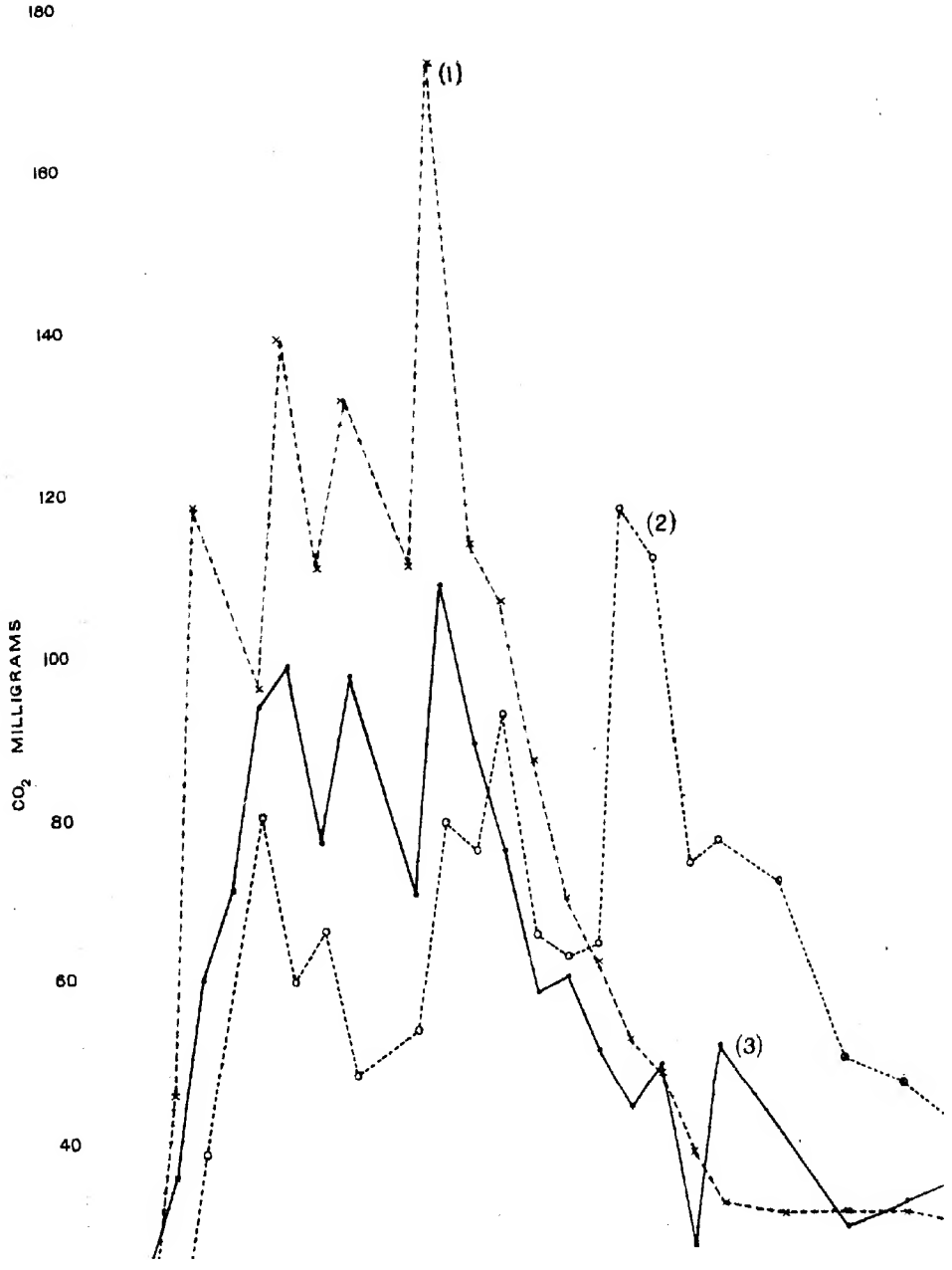
5 gms. of oilcake* and 100 grams of ignited sand were added to each lot. A pure culture of B. Subtilis was emulsified in water and 32 c.c. of the emulsion was added to each lot of soil; this was to make certain of the presence of a sufficiency of active bacteria in each sample, and to bring the water content up to the optimum.

The soils were then introduced into Woulff's bottles connected with aspirators, and air, freed from CO_2, was led over them and into Pettenkofer tubes containing standard Barium Hydroxide solution, and wash bottles containing standard acid for estimation of ammonia.

This method of arriving at a quantitative estimation of the bacterial activity of a soil has been found of great utility at Pusa, and its value as a means of determining the influence of varying treatment upon the soil complex will be very great, when a

* In all experiments involving use of oilcake the latter was extracted with Benzol to remove any residual oil and produce a more uniform source of organic nitrogen.

CURVE II



definite relationship has been established between the amount of CO_2 formed and the physiological activity of micro-organisms in the soil. So far as I have been able to determine, limiting factors for general bacterial activity, water, air, suitable temperature, are also limiting factors for CO_2 formation in soil; no CO_2 is formed in sterile soil, and in the case of thymol the partial suppression of vitality and the gradual recovery coincident with the volatilization of the antiseptic are well indicated by the CO_2 curve Table VI. Similarly no CO_2 is formed in perfectly dry soil, but rapid formation takes place on subsequent addition of moisture, and this in turn decreases on raising the proportion of water above the optimum required for bacterial activity in the soil. A further point will be noticed in studying the formation of CO_2 in soils *in vitro*, and that is the regular falling-off in gas production after a comparatively short period of two or three weeks; the maximum is generally reached within a few days and maintained for varying periods up to a week or ten days when ensues a sudden fall and a rapid decline to a minimum, which may continue apparently indefinitely. As this altered rate of production does not depend upon variation in the supply of water or of air, which may be kept constant throughout, nor upon failure of the food-supply, but closely resembles in kind the falling-off in physiological activity of a bacterial culture consequent upon the accumulation of the toxic products of its metabolism, it seems reasonable to conclude that we have here a measure of bacterial activity in the soil.

Curve II shews the differences in amounts of CO_2 formed during the period of observation, in the experiment described above, and it will be seen that the effect of toluene upon the CO_2 formation in this soil was greater in proportion to the amount applied, suggesting that this was due to its action either upon the physical condition or chemical constitution of the soil. All experiments made at Pusa with the CO_2 measurement method have demonstrated the close relationship between the amount of this gas formed and the quantity of air passed over the soil;

this point has been emphasized by Van Suchtelen (Cent. f. Bakt. II Abt. 28, p. 45) who has fully demonstrated the value of this method of estimating the biologic activity of a soil; it is difficult to avoid the conclusion that the addition of toluene to an arable soil increases its porosity to air, probably by removing in solution that portion of the humus, "the ether soluble residue," to which Greig Smith refers under the name of "Agricere," and thus increasing the pore space. At the same time the "water-proofing" effect of such waxy material upon the soil particles, as suggested by Greig Smith, would be diminished in proportion to the completeness of its removal, thus allowing of greater bacterial activity: I shall have occasion, in dealing with the biological analysis of tea-garden soils, to shew that there is some reason for supposing that one or more constituents of the organic matter of soils may exercise a depressing influence upon bacterial activity and that there are certain conditions obtaining in soils which result naturally in the formation and accumulation of such antiseptic substances. It is also possible that a considerable proportion of any toxins present in the soil would be removed together with this soil wax, but in any case, so far as the activity of soil bacteria as measured by their production of CO_2 is concerned, the action of toluene as shewn in this experiment can have no connexion with the protozoal content of the soil, the protozoa having been removed by the preliminary heating.

The close resemblance between the rate of CO_2 formation in soil and that of bacterial action in culture, and the fact that the latter is limited by the accumulation of toxic products of metabolism, lends further support to the hypothesis that bacterial action in soil is checked in a similar manner and by a similar cause.

Further experiments with this method of measuring bacterial activity by estimation of CO_2 resulting therefrom show an increase in the amount of the latter, corresponding in fact to concurrent increase in number of colonies, when plate cultures made with boiled soil extract are compared with similar plates with untreated extract. In one experiment two pairs of agar plates of

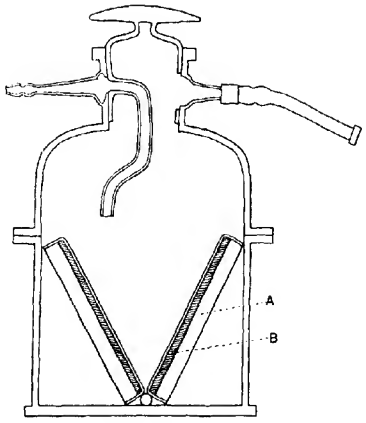


Fig. 2.

A. Inverted Petri dish.

B.—Pure Culture B. Prodigiosus on Agar.

B. Prodigiosus were used, one pair having been incubated with boiled and one pair with unboiled soil extract; the plates were placed in a Novy anaerobic culture apparatus and arranged as shewn in the drawing (Fig. 2); aspiration and measurement of CO_2 formation were carried on for five days. The results were as follows:—

Production of CO_2 by B. Prodigiosus cultures in the case of boiled and unboiled soil extracts.

TABLE XXXII.

Boiled.		Untreated.		Date.
Mgms. of CO_2 per diem.	Total.	Mgms. of CO_2 per diem.	Total.	
1·6	1·6	0·75	0·75	16th May.
10·4	12·0	5·6	6·35	17th ,,
9·3	21·3	8·15	14·5	18th ,,
3·6	24·9	4·2	18·7	19th ,,
2·65	27·55	4·1	22·8	20th ,,
2·4	29·65	1·65	24·45	21st ,,

It will be seen that the original difference due to variation in treatment and presumably consequent on the destruction of toxins in the soil water, is gradually diminished as a result of the multiplication of the organisms on the plates up to the limit of mutual interference.

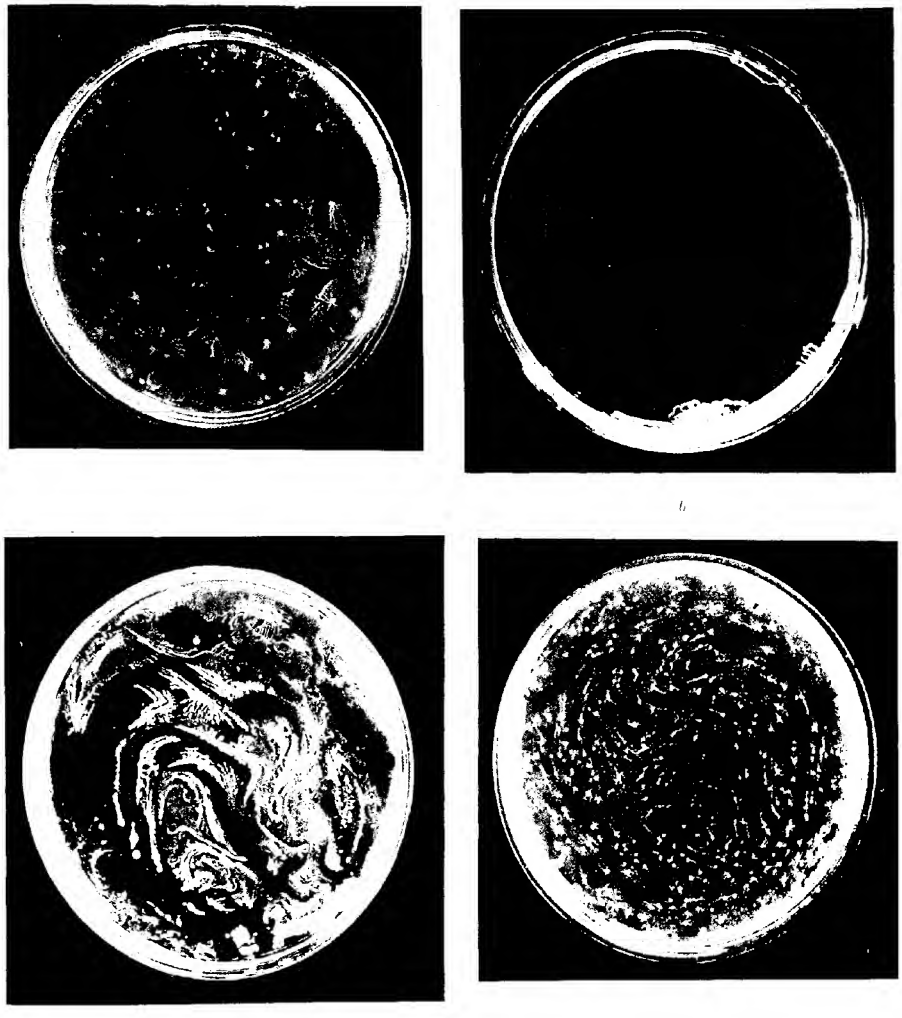
It is important to note that toxins may be produced in a soil extract by heating, in the same way as such are formed in a soil during sterilization by heat; thus, in the experiment described above, in which increased bacterial action resulted from boiling the soil extract, the soil used had been kept air-dry in the laboratory for some weeks; a further experiment in which a fresh sample of the same soil was used gave a precisely opposite result; no growth took place on the plates made from the boiled extract, whereas those from the unboiled extract produced a

normal number of colonies. Here the inhibition of growth was so complete that no room is left for doubt as to the presence of toxins in the soil extract, resulting from boiling, but not originally present in such quantity in the extract. It appears that the 15 minutes' boiling adopted in these experiments was sufficient to produce toxins from the organic matter in solution, but not sufficient to destroy them, which however would be effected by the longer period (one hour) given by Greig Smith (loc. cit.). In the case of air-dried soils the proportion between the soluble toxins present and other organic matter soluble in water might be such that boiling for 15 minutes would not only destroy the first but also any toxins formed by heating of the latter. Greig Smith shows that the amount of toxin, or at least the relative inhibitory power, remaining in a soil extract varies inversely with the length of time during which the extract was heated; this would confirm the view expressed above that the ultimate inhibitory effect depends upon the amount of toxin originally present, or upon the amount of material in solution in the extract which yields toxins on heating.

An experiment made with cultures of B. Prodigiosus incubated in a water extract of Pusa farm soil, which extract had been boiled for varying periods, shewed on plating an apparent increase in the amount of toxin present after boiling for a short time, and its eventual disappearance after further heating. Photographs of these plates are given in Plate VI. It is evident, therefore, that in dealing with such soil extracts from the point of view of their content of bacterio-toxins, it is important to take this factor into account, as otherwise contradictory results may be obtained, and, on the other hand, apparently concordant results might be misleading.

As has been pointed out by Russell (Journal of Agricultural Science, Part 2, Vol. III), heating some soils to 98°C. produces toxins which inhibit nitrification, whereas in the case of Pusa soil, autoclaving at 130°C. twice or thrice does not produce this effect, presumably on account of the subsequent dispersion or destruction of any toxins produced.

PLATE VI.



b

c

d

EFFECT OF VARYING HEAT ON SOIL TOXINS. PLATES, B. PRODIGIOSUS.

(*a*) Soil Extract.
(*b*) Soil Extract boiled one fourth hour.
(*c*) Soil Extract boiled half hour.
(*d*) ,, ,, one hour.

This line of enquiry has only recently been taken up at Pusa, so that further reference to the results obtained will not be made until fuller information is to hand.

It is difficult to avoid the conclusion that the increased fertility of the soil consequent on partial sterilization must depend not mainly upon any one, but upon a combination of causes, of which the elimination of phagocytes may be one and the destruction of bacterio-toxins another. In the case of "weathered" soils, it has been shown that this operation does not destroy all, or even most of the protozoa present, so that the protozoal theory cannot be called on to account for the results produced.

Lipman, Brown, and Owen (Cent. f. Bakt. II Abt. 30, Bd. No. 7/12—1911) have shewn that comparatively large quantities of ammonia are produced in highly aerated soils. An experiment with Pusa soil was carried out to determine the effect of aeration, such as occurs in "weathering," upon the rate of bacterial action, as compared with that in soil under ordinary conditions. For this purpose air was aspirated over soil containing varying proportions of sand, as in the experiments of Lipman, Brown and Owen above mentioned; the quantities of carbon dioxide and ammonia formed were determined by leading the air through standard solutions of Barium Hydrate and Sulphuric acid. Table XXXIII shews the amount of ammonia given off from the soil under these conditions during a period of 22 days.

Loss of Ammonia due to aeration of soil.

A. Pusa vegetable garden soil.

B. Pusa vegetable garden soil + 30% sand.

To 100 grams each of soils A + B, 0·938 gm. oilcake, (N = 0·06 % of soil weight) was added and water to make $\frac{1}{3}$ saturation.

Aspiration was carried on for 22 days and the ammonia given off estimated by absorption in $\frac{N}{20}$ sulphuric acid.

TABLE XXXIII.

NITROGEN LOST AS AMMONIA IN 22 DAYS.

A. 1·41 mgms. } From 100 gms. soil.
B. 2·59 „ }

It will be seen from this table that the increased aeration produced by adding 30 per cent. sand has resulted in nearly doubling the loss of ammonia; it appears probable that at least as much ammonia was formed in the undiluted soil, the loss of this gas in the diluted sample being due partly to aeration, and partly to lack of retentive power owing to the lower percentage of organic matter present. It will be seen that the actual loss of nitrogen as ammonia in the diluted soil over a period of three weeks would correspond in the field to as much as 77 lbs. per acre, *and although the field conditions might not approach those* of the experiment, a much smaller loss extending over a longer period would be of considerable moment in affecting the nitrogen supply of the soil.

Taking into consideration the relatively large amount of ammonia which must have been absorbed and retained by the soil in this experiment it is obvious that under the conditions of intensive aeration, extending over periods of several weeks, at the comparatively high temperatures which obtain in the operation of weathering, a large formation of ammonia with a corresponding loss of nitrogen may be expected and this loss would become rapidly greater, as the retentive power of the soil for ammonia diminished with the reduction of its organic matter. It is a question yet to be determined whether the nitrification proceeding concurrently with this ammonia formation is of sufficient intensity to fix the nitrogen set free from the organic matter by the ammonifying organisms under the conditions of field practice, but, judging from the loss of this element as ammonia observed in the above experiment, it appears probable that a similar loss takes place in weathered soil in the field, and that the increased fertility due to the rapid ammonification which takes place under these conditions, will depend for its

maintenance upon careful renewal of the organic nitrogen thus depleted.

The actual conditions obtaining in the field during the process of weathering, so far as moisture is concerned, may be considered to imply the existence of a series of soil strata containing varying amounts of water; those at and near the surface become practically air-dry, and contain eventually insufficient moisture either for nitrification or for the preliminary decomposition processes due to bacterial action; this has been ascertained by an experiment in which weathered soil containing 1·5—2% moisture was mixed with oil-cake and kept under analytical observation at intervals of seven days for a period of one month; no nitrate formation was detected during this time; further experiments shewed that no CO_2 or ammonia were *produced in air-dry soil, although, on subsequently adding water,* bacterial activity recommenced at the normal rate.

Below the upper "air-dry" layers of soil in the field is no doubt a series of others containing gradually increasing quantities of water, and at a certain point in the series nitrification would find its optimum moisture content, and so with ammonification and other activities of decay. The actual result of the frequent repetition of ploughing incidental to the method would be the gradual drying out of the soil and the consequent carrying down of the various optimal points deeper into the soil, the total effect of which would be the complete ammonification and probably nitrification of a very large proportion of the organic matter present. Another aspect of the case deserves consideration. As stated above, in air-dry soil there is no formation of nitrates, of ammonia, or of CO_2, and in fact we may conclude that the normal bacterial processes associated with the decay of organic matter are in abeyance; as a result of the repeated ploughing incidental to the process of "weathering," the top nine inches of the soil become practically air-dry, and consequently any organic matter contained in this stratum which has survived the processes of decay during its passage through the varying degrees of desiccation, will remain unchanged, so far as bacterial

action is concerned, until the water content is augmented. Here then we have a method of conservation balancing the rapid formation of ammonia and nitrates incidental to the early stages of the process, and beneficial in the sense that loss of nitrogen during this period will be greatly minimised. I have been able to show (Heeleaka Experiment Station Report, 1908) that in the use of organic manures for tea, rapid decomposition in the soil, of such material as oilcake, results in loss of crop, which may be minimised by a method of conservation depending upon the application of numerous small doses of manure instead of the normal single one; conservation of nitrogen in India may thus involve the avoidance of too rapid decomposition of organic manures, and what is good practice in a temperate climate might result in serious depletion of organic reserves at the higher temperatures obtaining in India.

The practice of "weathering" thus appears to place in the hands of the cultivator a means of rapidly converting the nitrogen of green manures, root residues, and cattle manure into ammonia and nitrates; the effect of this method upon the ultimate condition of the soil will depend upon the careful regulation of the ratio between the supply of organic matter and its depletion. Consideration of the rapid rise in the rate of formation of CO_2 with increased aeration will emphasize the need for replacement not only of nitrogen, but of carbon.

Further experiments are being carried out to determine the ultimate effect of continued weathering upon the constitution of the soil complex. Plate cultures made at intervals of seven days from artificially weathered soil show complete suppression of all anaerobes, as described above. As suggested above, the elimination of anaerobes might have a decidedly prejudicial effect upon the decomposition of cellulose elements in vegetable tissues and thus unfit the soil for green manuring and indeed for profiting by burial of weeds, stubble, and root residues, or even organic manures. Experiments are in progress to determine the relation between such alteration in the constitution of the bacterial flora and the ability of the soil to deal successfully with buried green manures.

It will be seen, therefore, that this method of cultivation depends upon the interaction of numerous complex factors, involving on the one hand the possibility of damage by depletion of organic nitrogen and carbon, and the alteration of the constitution of the soil flora, and, on the other, the rapid turnover of capital in the shape of organic nitrogen. I have made no specific reference to the effect of "weathering" upon water-supply to the crop, as this is a purely non-biological problem, but it is possible that this limiting factor may be affected by it to such an extent as to cause all biological agents to assume a position of relative unimportance.

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